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RELATION OF LENGTH OF PHOTOPERIOD AND INTENSITY OF SUPPLEMENTAL LIGHT TO THE PRODUCTION OF FLOWERS AND BERRIES IN THE GREENHOUSE BY SEVERAL VARIETIES OF POTATOES [1]

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INTRODUCTION

In order to conduct a breeding program for the production of new varieties of potatoes adapted to Nebraska conditions, it became necessary to devise some means of producing flowers and seed in the greenhouse during the winter, for in all parts of the State seed production in the field is undependable even with the most free-blooming varieties, and some varieties never bloom.

By using supplemental electric light until 11:30 p. m., Stevenson and Clark (*6*)[2] secured extensive blossom and berry production with 20 strains of potatoes (*Solanum tuberosum* L.) in the greenhouse at Arlington, Va., in March and April of 1932. Strains that had set seed naturally in the field in Maine bloomed more readily in the greenhouse than those that had not set seed.

Jones and Borthwick (*5*) concluded that "the first inflorescence of the potato was differentiated at approximately the same node under a range of conditions including size of seed piece, temperature and photoperiod." They also reported that flower primordia were developed in total darkness.

Clarke and Lombard (*3*) concluded that 16 hours was a suitable photoperiod at Beltsville, Md., for both flowering and fruiting with a number of potato varieties. However, with a 16-hour photoperiod the mean number of seed balls in the Triumph variety was only 2 per plant and such a free bloomer as Earlaine produced only 6.00 per plant. The supplemental light used had an intensity of only 20 to 30 foot-candles. The weakness of the supplemental light together with the cloudy winter days may account for the failure to obtain more berries on even the continuous-light plants, which with Earlaine averaged only 5.89 seed balls per plant. In view of their results and those of Jones and Borthwick (*5*), Clarke and Lombard concluded that the failure of flowers to develop in short photoperiods is due to the abscission of young buds.

Edmundson (*4*) reports that in the relatively bright winter days at Greeley, Colo., plants that emerged on January 10 or 12 in two winters bloomed and set seed in a constantly increasing quantity as

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[2] Italic numbers in parentheses refer to Literature Cited, p. 274.

the photoperiod was increased from 9 to 11, 13, and 17 hours. However, the maximum average number of seed balls for 10 varieties was only 6.15 per plant and the maximum percentage of pollinated flowers that produced seed balls was only 56. This low set might have been due to the 17-hour photoperiod being too short or the supplemental light (20 to 30 footcandles) being too weak, or both. Photoperiod did not alter the percentage of fertile pollen.

REVIEW OF SEED PRODUCTION IN NEBRASKA

The first attempt to produce potato seed in the Nebraska Experiment Station greenhouse at Lincoln was made in the winter and spring of 1937. The potatoes were planted the middle of January. The average light intensity outside the greenhouse during daylight hours for the entire period prior to the opening of the first blossoms in late March and early April, was about 300 gm.-cal. per square centimeter per day. This was supplemented with artificial light (about 25 footcandle intensity at the tops of the plants) to make a total photoperiod of 16 hours. Berries were produced by 32 lines of potatoes, flowers of which were fertilized with pollen from several good pollen-producing lines. Plants growing in gravel with a balanced nutrient solution produced more flowers and berries than those growing in a rich soil in ground beds. Many failures occurred when flowers were pollinated during the very hot days which occurred frequently after mid-April. Consequently few berries were obtained from the numerous flowers produced in late April and May.

In order to avoid the disastrous consequences of these very hot days in April and May, plantings were made on January 1 in 1938 and first blossoms were produced in early March. The mean daylight intensity for each month in gram-calories per square centimeter per day was as follows: January 190, February 243, March 333, and April 485. The mean light intensity for 40 days prior to the opening of blossoms was about 250 gm.-cal. (January 20–March 5). Supplemental light of about 25 foot-candles was used to make a 16-hour photoperiod. Under these conditions berries maturing during May were produced on 67 lines. This was still not early enough for the breeding program. Seed was needed that would germinate promptly when planted in mid-August and thus permit the seedling tubers to be harvested in February and complete their rest period in time for field planting in June.

Some of the writer's observations and the findings of Borthwick and Parker (*2*) suggested that long photoperiods might be used for only a relatively brief period prior to the induction of flower buds and also that more light during the photoperiod might improve flower production. During the winter of 1938–39, 12 varieties or clones of potatoes were planted on December 9, and the plants emerged during the period of shortest days, December 21 to 25 (*9*). Half of these plants were given supplementary light of 100 to 125 foot-candles at the plant tops for an 18-hour photoperiod and the other half only 30 to 40 foot-candles. The mean intensity of the natural daylight from emergence of plants to pollination of first flowers was 226.2 gm.-cal. per square centimeter per day. With the brighter supplemental light 3 varieties set berries quite readily, a fourth set very few, but 8 set none at all. With the weaker supplemental light only 2 varieties produced berries satisfactorily, 1 produced a very few, and the other

9 produced none. Another set of these lines was started on December 24, emerging about January 10. The daylight intensity from emergence to pollination of first flowers was higher than with the other set, namely, 254 gm.-cal. per day. The supplemental light for the 18-hour photoperiod was only of 30 to 40 foot-candle intensity. With this slightly brighter daylight but weak supplemental light, 4 varieties produced berries in a fairly satisfactory manner and 6 of the 12 varieties produced some berries. The results with these 3 sets of plants indicated that a long photoperiod was not in itself sufficient for flower and berry production, but that more light during the photoperiod either from brighter sunshine or brighter supplemental light was necessary.

In 1938–39, when supplemental light of 30 to 40 foot-candle intensity was used, extensive blooming occurred with a 24-hour photoperiod, but a 16-hour photoperiod was not satisfactory and a 13-hour photoperiod was very inadequate for even the most free-blooming varieties. With these same plants a high level of nitrogen nutrition was necessary for best flower and pollen production.

With early strain Triumph plants a few flowers were produced when a 16-hour photoperiod (20 to 30 foot-candles of supplemental light) was used for 35 days following plant emergence on February 1, 1939. When 16-hour days were used for less than this time, none of the flowers attained maturity and most of them dropped off. Either these days were too short or light was too weak to bring about floral induction.

In the winter of 1939–40 early and midseason strains of Triumph potatoes planted on December 29 were supplied with 18- or 24-hour photoperiods during intervals increasing by 10 days from 20 to 70 days after the sprouts had been started in the light in a humid chamber (*8*). In a parallel series sprouting occurred in the dark (in sand), and supplemental light which started 10 days after plant emergence was continued for 10 to 40 days. The midseason strain produced berries more readily than the early one, and both produced more with 24- than with 18-hour photoperiods. Best berry production occurred when long photoperiods were used continuously until the first few flowers were pollinated. Long days during the last few weeks prior to blooming appeared to be essential for satisfactory berry production. Long photoperiods during the sprouting period and first few days thereafter appeared to exert some beneficial effect measurable as increased numbers of berries. The supplemental light used was between 100- and 200-foot-candle intensity at the tops of the plants.

These earlier endeavors indicated quite clearly that either greater light intensity or longer days must be used in order to obtain berry production during the winter with most varieties or lines of potatoes. The work during the winter of 1939–40 was planned to determine the effect of greatly increased light intensity during 18- and 24-hour photoperiods (throughout all or part of the life of the plant) upon the production of berries both by varieties that produce berries easily and by those that seldom fruit.

EXPERIMENTAL METHODS

The four varieties selected and some of the specific flowering characteristics considered in choosing them were the following:

Earlaine.—Weakly vegetative early variety that produces numerous fertile flowers when most other varieties drop their flowers or produce only primordia.

Triumph 12.—The earliest clonal strain of Triumph potatoes that has been selected in Nebraska. It does not produce flowers unless conditions are very favorable, and generally it produces little or no pollen, although at several earlier times pollen suitable for crossing and selfing has been obtained from it. No pollen was produced by this strain in any treatment of this experiment. Abscission of partly developed flowers was most common with this clone. It does not bloom as freely as the later maturing clonal lines of Triumph potatoes.

B4–1.—A hybrid secured from Dr. F. A. Krantz and listed by him as Minnesota 29.32–1–34. It blooms quite readily and produces more pollen per anther than almost any other variety. The percentage of viable pollen was found to be somewhat lower than that of Katahdin, but this is of little consequence in view of the volume of pollen produced.[3]

Katahdin.—This variety produces an abundance of flowers with excellent pollen under a wide range of conditions.

Seed pieces of early harvested tubers were planted in sand on November 1, 1939. On November 21, 1939, when most sprouts were 2 to 5 cm. long, the sprouting seed pieces were carefully removed, sorted as to size, and held in shallow basins of water until replanted. Two sprouting seed pieces were planted in fine gravel in a 10-inch pot. One seed piece placed to the south was covered only 1 cm. in order to facilitate the later removal of stolons and tubers so as to retain carbohydrates in the tops. However, blooming was so satisfactory in the better treatments that tuber removal was not resorted to. The north seed piece in each pot was covered to a depth of 10 cm. The tops of most of these north sprouts were up to or above the surface by November 25, which is considered as the emergence date. Six pots of two seed pieces each were planted with each of the four varieties in each of eight light treatments. The pots of one treatment were arranged in three east to west rows with eight pots per row. All pots were placed tightly against each other. A 20-inch space was provided between treatments. Because of the crowding of the plants within one treatment and the consequent unequal illumination of all but the upper leaves, the plants of each variety were placed in a different position in each row. The positions of the plants of each variety were as follows:

North row	B T E K B T E K
South row	E K B T E K B T
Center row	T E K B T E K B

(E, Earlaine; K, Katahdin; B, B4–1, and T, Triumph 12.)

The gravel in the pots was flooded one to three times a day, according to the amount of transpiration, by raising a 5-gallon reservoir of nutrient solution connected to the pots with tubes in the bottom of each pot. After about 30 minutes the gravel was flooded and the reservoirs were lowered to a level sufficient to maintain about 2 inches of solution in the bottom of each pot. This level was maintained by adding sufficient water daily to fill all pots completely when the reservoirs were raised. Nutrient solution was made up from commercial salts according to the formula listed as 2D by Withrow and Biebel, (*10*), 45 gm. of the dry mixed salts being used to each 5 gallons of water. The solution was drained and renewed once each week. Solution reservoirs were covered with boards to reduce evaporation and prevent contamination by algae (fig. 1).

Three degrees of supplemental light intensity were secured by using 300-, 150-, and 60-watt bulbs. These bulbs were arranged over each

[3] CLARKE, A. E. RELATION OF NITROGEN SUPPLY AND DAY LENGTH TO FLOWER PRODUCTION AND POLLEN FERTILITY IN POTATO VARIETIES. Ann. Rpts. Coop. Projects, 1938–39 and 1939–40. [Unpublished.]

treatment on a framework that supported two rows of five bulbs each. The bulbs were suspended 2 feet above the pots when the experiment was started. They were raised at intervals as the plants grew so that they remained at this distance above the tops of the plants with a reasonable degree of constancy. In order to utilize electricity most efficiently, each bulb was provided with a circular reflector the inside

FIGURE 1.—Plants used for treatment 8, showing method of growing plants in gravel in 10-inch pots with nutrient solution supplied from 5-gallon cans, through tubes entering the bottoms of pots; method of arranging lights so that they can be raised as plants grow; position of thermograph; black curtains for separating light treatments (one curtain drawn); and white sheets of paper used for several weeks to reflect light on to plants. Panels along side bench were made of sisal kraft paper painted with aluminum paint and were raised at night (as shown) to protect seedling plants of another project from the long photoperiod. The 5-gallon cans were raised on to the trestle to flood pots with nutrient solution and were then lowered so that nutrient solution was maintained at a depth of about 2 inches in the bottom of the pots. One can supplied eight pots. Cans were covered with a board to prevent growth of algae in the solutions.

of which was painted with aluminum paint (this reflected approximately 10 percent more light than white lead paint). In the earlier weeks white sheets of paper were suspended from the sides and ends of the light-supporting frames so as to reflect light back on to the plants and also to prevent absorption by the black dividing curtains. These devices increased the light from 40 to 60 percent. As the plants grew, the use of these sheets of paper became complicated and only the circular reflectors were continued. Mean values for this supplemental light, as reported in table 1, were secured from readings made with a Weston meter at the tops of the 48 plants of each treatment. Readings were made each time just before and just after the lights were raised. In the course of the experiment 145 sets of light readings or almost 7,000 individual readings were secured. The occasional very high mean light readings recorded were due to the fact that the tips of a few plants grew very close to the bulbs. With some plants

located between the lights the readings were low before the lights were raised because the tops grew above the zone of bright light. Very few flowers developed after February 1 when the last readings were made.

In order to secure photoperiods of the definitely desired lengths, lights were turned on at 7 a. m. and left on till the daylight was brighter than that coming from the electric lights, which was generally between 8:30 and 9:30 a. m. On several very dark days the lights were left on throughout the day. On most days only the brightest light treatment gained by such midday supplemental light, and the weakest light treatment gained only during the dim-light portions in the morning and evening. In the late afternoon, lights were generally turned on between 4:30 and 5 p. m. For the 18-hour photoperiod lights were automatically turned off at 1 a. m. Black curtains were drawn between treatments involving either different light intensities or different photoperiods. These curtains were drawn across when lights were turned on in the late afternoon, but in the morning when the lights were left on to supplement weak daylight, the curtains were drawn back.

TABLE 1.—*Photoperiod, mean light intensity, and mean temperature used with various treatments*

Treatment No.	Photoperiod	Power of each of 10 light bulbs used per treatment	Mean supplemental light intensity, Nov. 23 to Feb. 1	Initiation or period of supplemental light	Mean temperature at tops of plants, Nov. 24 to Mar. 30
	Hours	*Watts*	*Foot-candles*		°*F.*
1	24	300	481	Nov. 21	63.9
2	24	300	493	Nov. 1	
3	24	300	501	Nov. 21–Dec. 18	
	18	60	72	Dec. 19–Jan. 6	
	24	300	509	Jan. 7–Jan. 15	
	9.5–12.4		0	Jan. 16	
4	24	150	216	Nov. 21	63.6
5	18	150	200	do	
6	18	300	515	do	62.9
7	24	60	65	do	
8	24	60	58	Nov. 1	57.5

Because of the great amount of heat generated by the large light bulbs, it was impossible to maintain uniform temperatures for all treatments. In order to secure similar temperatures, fans were suspended just below the bulbs at one end of each of the light frames supporting 300-watt bulbs. By blowing the air from the cooler side of the greenhouse across the tops of the plants, the average temperature there was lowered 6° to 10°. As a result of the extensive ventilation which was necessary at night to maintain a generally low temperature in the greenhouse, the temperature in the vicinity of the weaker lights averaged about 6° F. lower than that under the brighter lights. As the day temperatures were almost the same in all parts of the greenhouse, these differences in the temperature for the entire period resulted from the higher temperatures that occurred during the supplemental lighting period. Daytime maximum temperature never exceeded 70° on cloudy days and exceeded 75° only during a few days of bright sunshine. Thermographs were suspended from the light frames of four treatments so that they recorded the temperature near the tops of the plants and always at the same distance below the lights. The mean daily and weekly temperatures were secured from thermograph sheets by means of a planimeter. The weekly means are shown in figure 2, and the seasonal means in table 1.

With daily supplemental lighting, the most extensive flower development occurred in late December and early January when the natural day length was only between 9.2 and 9.6 hours and the daily light intensity, registered a short distance from the greenhouse (*1*), averaged between 145 and 210 gm.-cal. per square centimeter during the various weeks (fig. 3). Under these conditions other potato plants of a num-

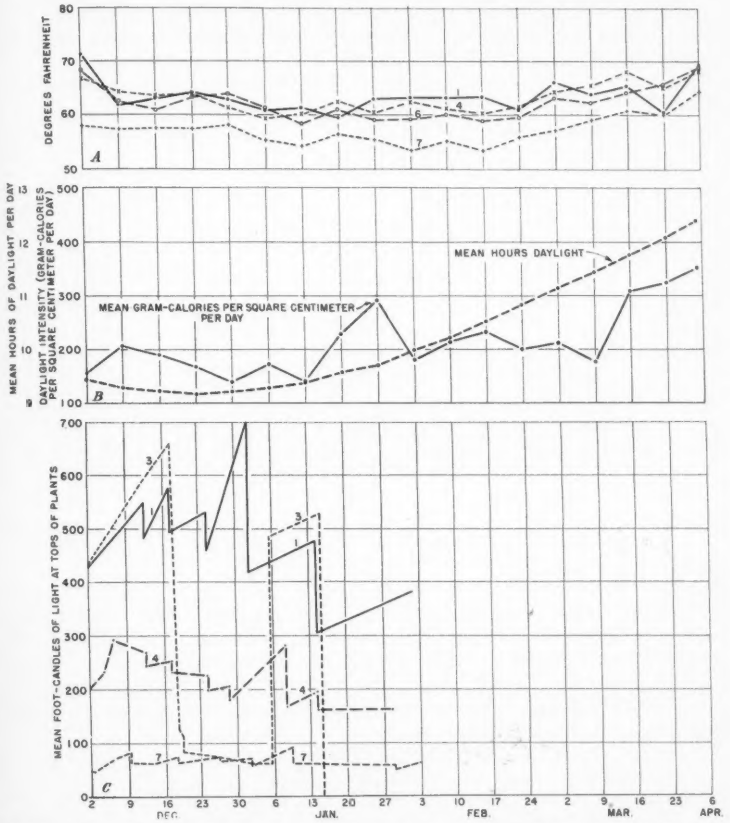

FIGURE 2.—Mean temperature, by weeks, in the vicinity of the tops of plants of each of four treatments described in table 1 (*A*), mean hours of daylight and weekly mean gram-calories per square centimeter per day represented by the daylight intensity (*B*), and the intensity of the supplemental light (foot-candles) at the tops of plants of each of four treatments listed in table 1 (*C*).

ber of varieties growing in the same greenhouse without supplemental light were typical of short-day plants described in an earlier paper (*7*); i. e., they had a single axis without axillary shoots and the inflorescence never developed beyond the primordial stage.

Every flower was pollinated at or just before anthesis with pollen from flowers of the three pollen-producing varieties; the pollen used was from flowers in the same light treatment. All relevant informa-

tion was noted on the tags attached to each flower at the time of pollination. Notes concerning abortion of flowers or degree of development of primordia were recorded for each cluster when the developing berries were bagged. Berries were harvested when ripe, during March and April. All seed was weighed by clusters after having been extracted by fermentation, washing, and drying.

Some estimate of the vegetative growth was secured by measuring the height of each plant a few days after it emerged and at weekly intervals until February 7, when the growth of the third axis was complete with practically all plants.

Just prior to the termination of the project an effort was made to determine relative foliage development. Four terminal leaflets from each plant were removed, and the leaf outlines were traced on sheets

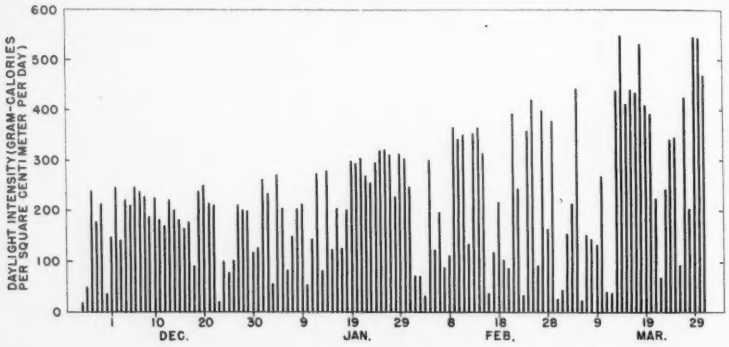

FIGURE 3.—Intensity of natural daylight at Lincoln, Nebr., in gram-calories per square centimeter per day for each day of the experiment, 1939–40.

of paper placed on a translucent glass over a light. Later the leaf areas were calculated by means of a planimeter. After the berries were harvested the plant tops were cut off at the surface. The length and number of nodes in each axis and in the underground stem parts were recorded for each plant. The number of tubers weighing more than 5 gm. and the total number and weight of tubers were recorded by plants.

EXPERIMENTAL RESULTS

VEGETATIVE GROWTH

A brief consideration of the nature of the vegetative growth is essential for understanding the differences in blooming habits of the varieties under the different treatments. Plants grown either with the lowest supplemental light intensity or the longest photoperiod (24 hours) made the most rapid growth of any and grew to be the tallest plants (fig. 4). The reduction in growth rate when berries were developing was most noticeable with the plants grown in a 24-hour photoperiod with the brighter light—the treatment that produced the heaviest crop of berries (treatment 1). In contrast, plants grown with the weakest supplemental light continued to grow at a rapid rate practically all season (treatment 7).

The length of each axis increased as the supplemental light intensity decreased (table 2, fig. 5). A reduction in length of photoperiod

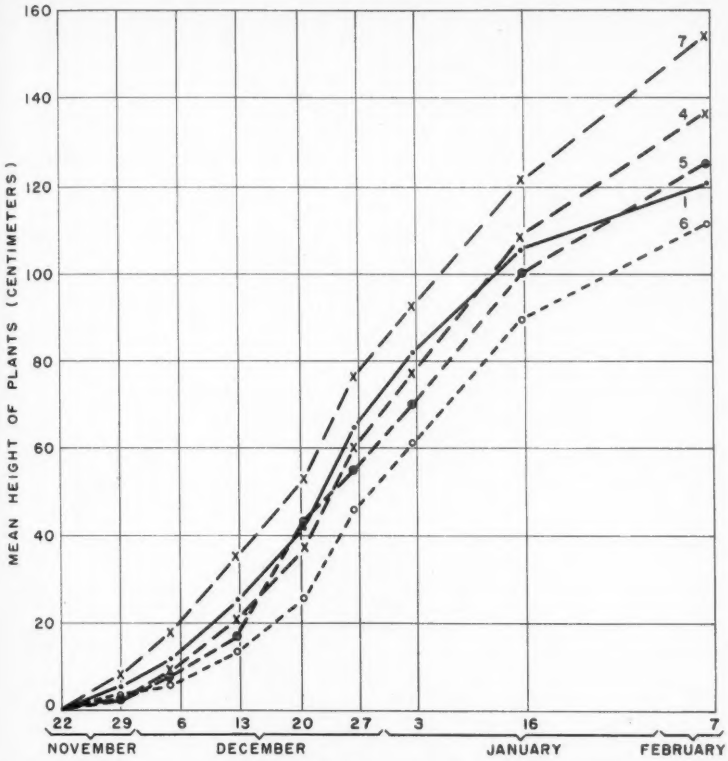

FIGURE 4.—Mean height of plants of all varieties in each of five treatments listed in table 1 at various dates till vegetative growth was terminated at the third axis by pruning.

brought about little difference in the central axes, but caused lateral axes to be much shorter. The lateral axes were less than half as long as the central axes. The 18-hour photoperiod with brightest light (treatment 6) produced the shortest plants. These plants were darker and more uniform than those of any other treatment (fig 5). Linear stem growth was greatest in Katahdin, followed in order by B4–1, Triumph 12, and Earlaine.

The mean size of the apical leaflets was found to increase with each decrease in light intensity or decrease in length of photoperiod (table 3). The leaflet area of various varieties arranged in increasing order was as follows: B4–1, Katahdin, Triumph 12, and Earlaine.

TIME OF BLOOMING

Within the range of this experiment, time of flower production was advanced more by increasing the intensity of the supplemental light than by extending the photoperiod (fig. 6). The development of sprouts in light with a 24-hour photoperiod hastened blooming in Earlaine, B4–1, and Katahdin, but delayed it in Triumph 12 when

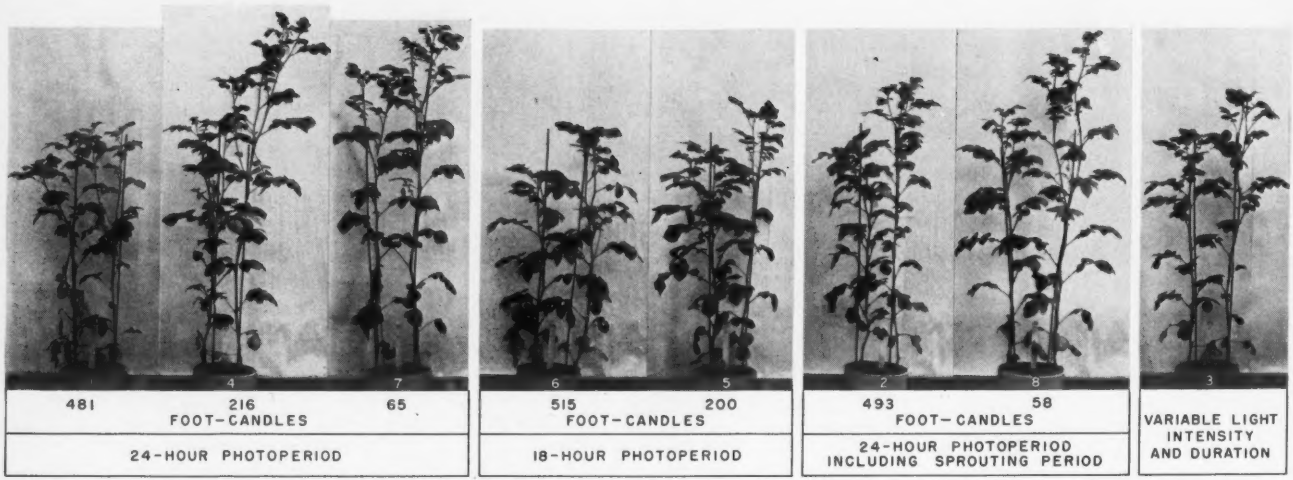

FIGURE 5.—One Triumph 12 plant from the north row under each light treatment listed in table 1 as it appeared on January 20.

the brightest light, was used (treatment 2 vs. treatment 1). With the weakest light blooming was delayed in all varieties (treatment 8 vs. treatment 7). Under the latter condition the status of Triumph 12 was uncertain because of the small number of blossoms with both treatments.

TABLE 2.—*Mean length of axes of the plants of all varieties in each treatment*

Treatment No.	Photoperiod	Mean supplemental light intensity, Nov. 23 to Feb. 1	Length of—			Total length
			Central axis	First lateral	Second lateral	
	Hours	*Foot-candles*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
1	24	481	75	37	41	153
4	24	216	86	41	47	174
7	24	65	100	42	48	190
6	18	515	75	28	29	132
5	18	200	86	33	33	152
2[1]	24	493	80	31	36	147
8[1]	24	58	87	45	45	177
3[2]	(2)	(2)	80	34	40	154
All treatments			84	36	40	160

[1] Sprouts developed in 24-hour photoperiod of given light intensity.
[2] Photoperiod and light intensity altered during life of plant, see table 1.

TABLE 3.— *Mean area per leaflet of systematically chosen apical leaflets from plants of all varieties in each treatment*

Treatment No.	Photoperiod	Mean supplemental light intensity, Nov. 23 to Feb. 1	Mean area per leaflet for—				Mean, all varieties
			Earlaine	Triumph 12	B4-1	Katahdin	
	Hours	*Foot-candles*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
1	24	481	27.3	22.1	19.4	20.6	22.3
4	24	216	29.9	32.3	23.8	26.6	28.1
7	24	65	37.5	32.5	29.2	29.1	32.1
6	18	515	31.6	30.7	26.6	31.7	30.2
5	18	200	35.1	33.7	28.3	33.5	32.8
2[1]	24	493	31.4	22.3	20.6	20.8	23.7
8[1]	24	58	28.0	31.1	27.2	29.5	29.0
3	(2)	(2)	27.6	28.3	24.3	23.8	26 0
Mean			30.9	29.3	24.9	27.0	28.0

[1] Sprouts developed in 24-hour photoperiod of given light intensity.
[2] Photoperiod and light intensity altered during life of plant, see table 1.

The mean dates of pollination for the flowers of all varieties produced in a 24-hour photoperiod in the order of decreasing light intensity were January 14, 21, and 25. The mean pollination dates for bright and intermediate light with an 18-hour photoperiod were January 20 and 23 respectively.

NUMBER OF PRIMORDIA AND FLOWERS

Fewer primordia and flowers were developed in the second than in the first inflorescence, and still fewer were developed in the third. The number of undeveloped primordia was about the same in all inflorescences, but the number of flowers diminished rapidly with each succeeding inflorescence.

The number of discernible floral primordia differentiated by three inflorescences per plant increased noticeably with a 24-hour photo-

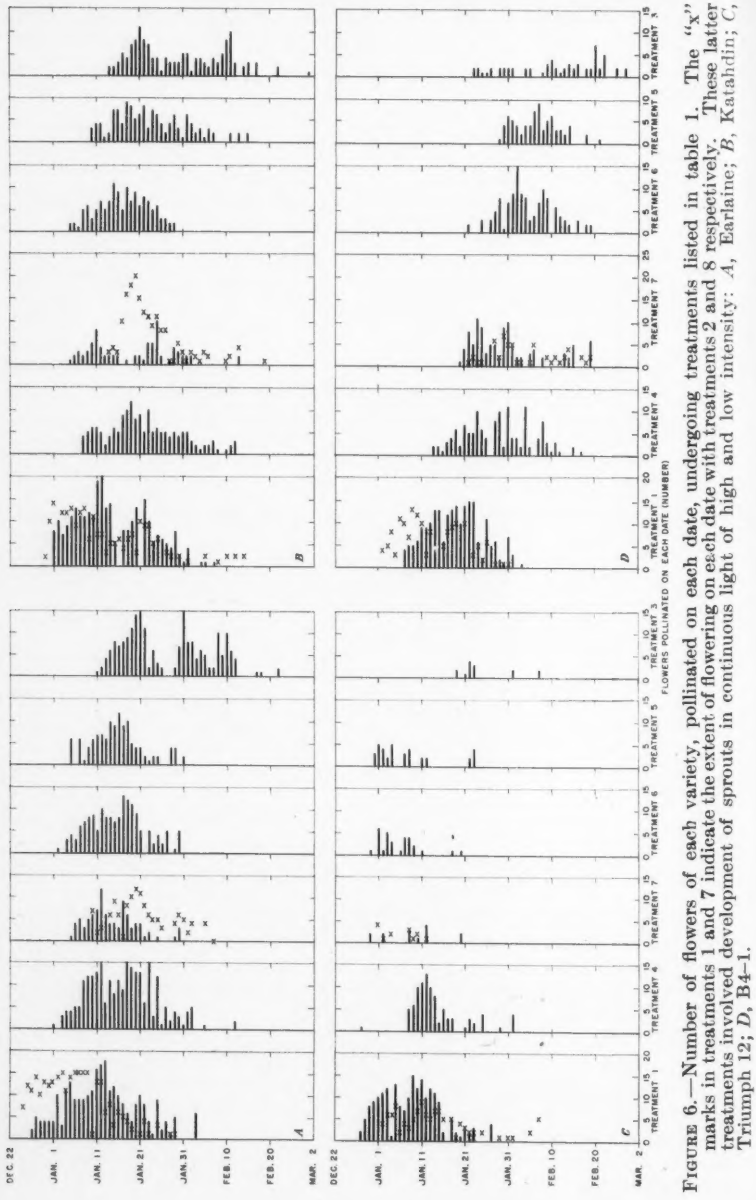

FIGURE 6.—Number of flowers of each variety, pollinated on each date, undergoing treatments listed in table 1. The "x" marks in treatments 1 and 7 indicate the extent of flowering on each date with treatments 2 and 8 respectively. These latter treatments involved development of sprouts in continuous light of high and low intensity: *A*, Earlaine; *B*, Katahdin; *C*, Triumph 12; *D*, B4-1.

period, but was not altered appreciably by differences in light intensity (table 4). The number of primordia in the first inflorescence of each plant was generally smaller with the lowest than with the highest supplemental light used, having averaged 11.7 per cluster for the former and 13.5 for the latter. Fewer primordia failed to develop to flowers as the light intensity was increased (table 4). Primordia abortion generally decreased slightly as the photoperiod increased.

The number of flowers sufficiently developed for pollination was greatest when the brightest supplemental light was used for the 24-hour photoperiod (table 4). The effectiveness of the 24-hour photoperiod in producing good flowers was greatly enhanced by using the brightest light. A 24-hour period with weak supplemental light was relatively ineffective for floral production. In earlier work light intensities of 30 to 40 foot-candles used to make 16- to 18-hour photoperiods were found to be still more inadequate in producing flowers.

With the scant-blooming Triumph 12, the number of flowers produced by using the brightest light in a 24-hour photoperiod was so much greater that all other treatments appeared relatively ineffective. With free bloomers such as the other three varieties, supplemental light of half the intensity was relatively effective with a 24-hour photoperiod. However, with the 18-hour photoperiod, reasonably satisfactory flower production occurred only with Earlaine and Katahdin. These two varieties also bloomed quite well in the variable light treatment (treatment 3).

TABLE 4.—*Mean number of floral primordia differentiated, primordia dropping, flowers pollinated, berries harvested, and mean weight of berries and of seed per plant of each variety in each treatment*

TOTAL NUMBER OF FLORAL PRIMORDIA DIFFERENTIATED

Treatment No.	Photoperiod	Mean supplemental light intensity, Nov. 23 to Feb. 1	Total on 3 clusters, mean of 1 plant for—				All varieties
			Earlaine	Triumph 12	B4-1	Katahdin	
	Hours	*Foot candles*	*Number*	*Number*	*Number*	*Number*	*Number*
1	24	481	29	29	24	31	28.2
4	24	216	38	26	24	30	29.5
7	24	65	29	24	27	25	26.2
6	18	515	26	21	21	27	23.7
5	18	200	24	24	21	28	24.3
2[1]	24	493	29	26	27	30	28.0
8[1]	24	58	25	23	25	32	26.2
3	(2)	(2)	29	27	21	32	27.3
All treatments			28.6	25.0	23.8	29.4	26.7

TOTAL NUMBER OF PRIMORDIA DROPPING

1	24	481	9	13	8	9	9.7
4	24	216	19	20	13	17	17.3
7	24	65	22	23	18	19	20.5
6	18	515	14	19	13	15	15.2
5	18	200	14	22	15	18	17.3
2[1]	24	493	10	17	13	11	12.8
8[1]	24	58	16	21	16	18	17.7
3	(2)	(2)	14	26	17	22	19.8
All treatments			14.8	20.1	14.1	16.1	16.3

TABLE 4.—*Mean number of floral primordia differentiated, primordia dropping, flowers pollinated, berries harvested, and mean weight of berries and of seed per plant of each variety in each treatment*—Continued.

TOTAL NUMBER OF FLOWERS POLLINATED

Treatment No.	Photoperiod	Mean supplemental light intensity, Nov. 23 to Feb. 1	Total on 3 clusters, mean of 1 plant for—				All varieties
			Earlaine	Triumph 12	B4–1	Katahdin	
1	24	481	20	16	16	22	18.5
4	24	216	19	6	11	13	12.2
7	24	65	7	1	9	6	5.7
6	18	515	12	2	8	12	8.5
5	18	200	10	2	6	10	7.0
2[1]	24	493	19	9	14	19	15.2
8[1]	24	58	9	2	9	14	8.5
3	[2]	[2]	15	1	4	10	7.5
All treatments			13.8	4.9	9.6	13.4	10.4

TOTAL NUMBER OF BERRIES PRODUCED

1	24	481	15.8	13.7	12.3	17.8	14.9
4	24	216	17.5	4.8	9.5	11.5	10.8
7	24	65	6.4	.5	5.1	7.4	4.8
6	18	515	10.4	1.2	7.5	11.4	7.6
5	18	200	8.3	1.1	4.8	9.1	5.8
2[1]	24	493	15.8	1.7	11.6	16.9	11.5
8[1]	24	58	7.7	1.0	8.3	10.3	6.8
3	[2]	[2]	12.0	.7	2.5	6.9	5.5
All treatments			11.7	3.1	7.7	11.4	8.5

WEIGHT OF BERRIES

			Grams	*Grams*	*Grams*	*Grams*	*Grams*
1	24	481	111.6	109.5	78.2	147.6	112.3
4	24	216	122.5	42.3	66.0	84.8	78.9
7	24	65	42.1	5.3	34.0	65.5	36.7
6	18	515	74.8	9.8	53.0	87.7	56.3
5	18	200	53.3	8.8	41.8	60.3	41.0
2[1]	24	493	114.3	49.9	66.0	123.8	88.5
8[1]	24	58	46.5	7.8	58.8	84.3	49.3
3	[2]	[2]	81.2	5.2	17.3	59.2	40.7
All treatments			81.0	29.9	51.9	89.2	63.0

AIR-DRY WEIGHT OF SEED

1	24	481	1.94	1.67	.98	1.84	1.60
4	24	216	2.16	.62	.75	1.06	1.15
7	24	65	.64	.09	.48	.71	.48
6	18	515	1.19	.12	.60	.98	.72
5	18	200	.78	.14	.49	.76	.55
2[1]	24	493	1.83	.75	.79	1.59	1.24
8[1]	24	58	.83	.13	.75	1.05	.69
3	[2]	[2]	1.39	.08	.19	.66	.58
All treatments			1.34	.45	.63	1.08	.88

[1] Sprouts developed in 24-hour photoperiod of given light intensity.
[2] Photoperiod and light intensity altered during life of plant: see table 1.

In all varieties some pollinated flowers failed to develop into berries. These failures are attributed to the fact that although pollinated before the corolla opened and once or twice thereafter (with all flowers present), some of the flowers abscissed. In some instances, especially in very large inflorescences, the development of the berries from the latest flowers was inhibited by the rapid growth of berries that were initiated earlier. The loss of flowers at this stage was much smaller than prior to the period of anthesis. The percentage of pollinated

flowers that dropped off without producing berries increased with each succeedingly later inflorescence. The number dropping was much the same with all varieties (difference between fourth and third sections of table 4).

The actual number of flowers dropping varied by treatments and on a percenatge basis it was much greater with the shorter photoperiod or lower light intensity.

NUMBER OF BERRIES

The number of berries per plant decreased greatly as the supplemental light intensity decreased or the photoperiod was shortened (table 4). With Earlaine and Katahdin reasonably satisfactory berry production took place with the two brightest light intensities and with both photoperiods. However, in the case of Triumph, the only acceptable treatment for berry production was the 24-hour photoperiod with brightest supplemental light, and with this treatment the berry production of Triumph compared very favorably with that of Earlaine and Katahdin (fig. 7). The B4–1 line was almost as dependent on abundant illumination as the Triumph. Earlaine exhibited the most versatility for blossom and berry production as it produced a good crop of berries with the variable light treatment (treatment 3) with which the berry crops of Triumph and B4–1 were practically failures. Apparently bright light is needed until flowers are well developed if a satisfactory crop of berries is desired. Starting sprouts in continuous light (treatments 2 and 8) did not materially influence the number of berries. About three-fourths of the berries were produced in the first clusters. With the least fruitful treatments no berries were produced in third clusters of some varieties. In fact, in such cases the third inflorescences were very poorly developed.

WEIGHT OF BERRIES

The total weight of berries was altered by treatments in much the same manner as total number of berries, having been increased by increases in both photoperiod and light intensity (table 4). The size of individual berries varied greatly and no differences in mean weight per berry could be established for treatments. However, differences in size of berries produced by different varieties were relatively great. The mean weights of berries in all treatments of each variety (calculated from the fourth and fifth sections of table 4) were as follows:

Variety	*Weight in grams*
Triumph	9. 66
Katahdin	7. 82
Earlaine	6. 92
B4–1	6. 74

AIR-DRY WEIGHT OF SEED

Again, with each decrease in light intensity or duration of photoperiod there was a pronounced decrease in the weight of seed produced by each treatment (table 4). With the light of greatest intensity during a 24-hour photoperiod, plants of the four varieties produced two to more than three times as much seed as those with the weakest light. Increasing the length of the photoperiod from 18 to 24 hours more than doubled the weight of seed. The longer day increased the weight of seed relatively more than it did the weight of the berries.

The brightest light treatment with a 24-hour day exerted the most pronounced influence with the Triumph, the seed weight of which was increased about twentyfold above that of the weakest light. With

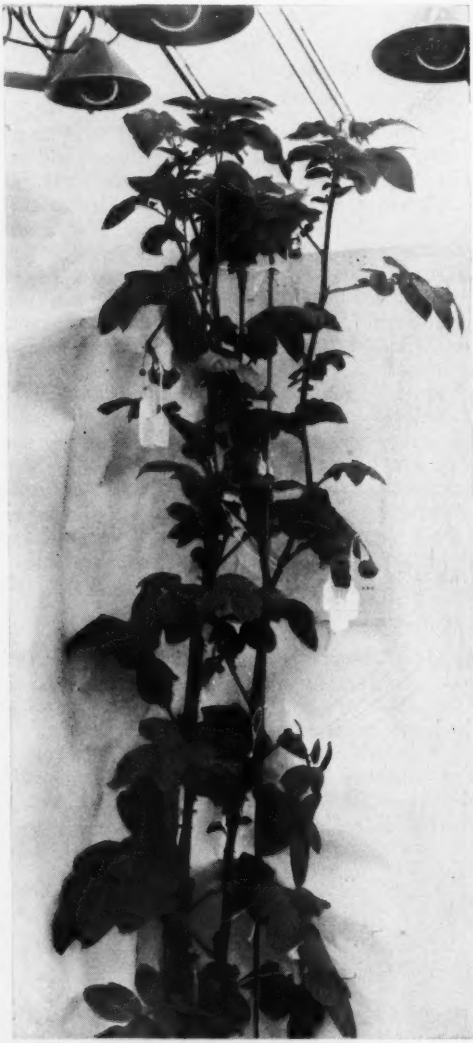

FIGURE 7.—Two Triumph 12 plants which underwent treatment 1 described in table 1, showing type of vegetative growth and several clusters of berries.

the variable light (treatment 3), seed production was fair with Earlaine, poor with Katahdin, and very poor with Triumph and B4–1.

There was more difference between varieties in the weight of seed per weight of berry than between treatments, Earlaine having the

greatest seed weight per berry weight and Triumph next; between the other two varieties there was little difference (calculations made from the fifth and sixth sections of table 4).

TUBER PRODUCTION

With the low temperatures of this experiment good tuberization occurred with all treatments. The mean number of tubers produced in all treatments was 8.7 per plant and the mean total weight was 241 gm. Differences between treatments were too small and irregular to warrant comment. The most significant difference was between varieties. Triumph consistently produced the greatest number and weight of tubers and Earlaine the smallest. The average production per plant for all treatments was 327 gm. with Triumph and 143 gm. with Earlaine.

GENERAL CONCLUSIONS

As a result of this work and previously published work which is cited, it appears that a long photoperiod is essential to induce satisfactory seed production during the winter with the Triumph variety and others that do not bloom readily. Seed production is improved by using relatively intense supplemental light. This increased intensity of supplemental light is more important when the natural daylight is inferior in quality and of short duration. Bright light and a long photoperiod must be used throughout the life of the plant, or at least during the greater part of it. Stated in another way—it seems that a minimum daily amount of light is needed throughout the life of the plant for flower and seed production and if much of this occurs as natural daylight, less need be applied supplementally. Furthermore, while a long photoperiod appears highly desirable, it can be compensated to a considerable extent by an increase in light intensity. A very long photoperiod with very bright supplemental light can be depended upon to produce flowers and seed.

SUMMARY

The experiment herein reported was planned to determine the relative effectiveness for the production of potato seed in the greenhouse, of different intensities of supplemental light with 18- and 24-hour photoperiods, of intermittent use of long photoperiods, and of the sprouting of parent seed tubers in continuous light.

The most extensive blossom, berry, and seed production took place when a light intensity of about 500 foot-candles at the tops of the plants was used for supplemental light during a 24-hour photoperiod. This treatment was effective with varieties that do not bloom or set fruit readily. The amount of blooming and berry production with all varieties decreased as the photoperiod or light intensity decreased.

The effectiveness of a 24-hour photoperiod for increasing the number of berries was greatly enhanced by a little more than doubling the supplemental light intensity, i. e., increasing it from about 200 to about 500-foot candles. When the intensity of natural daylight was low, the use of supplemental light of only 60 foot-candles intensity in a 24-hour photoperiod was practically of no value for producing seed with varieties that do not bloom very readily.

When long photoperiods of bright supplemental light were used early in the life of the plant and supplemental light was used only at

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intervals thereafter, blooming took place better with varieties that bloom easily than when weak supplemental light was used every day, but this method was practically a failure with varieties that do not bloom readily.

The number of primordia, blossoms, and berries was greatest on the first clusters, least on the third or last. The degree of development of the second and third clusters was dependent upon increase in day length and light intensity.

The production of sprouts in continuous light exerted some influence on time of flowering, but did not affect the production of berries.

The vegetative growth was most rapid and extensive with the plants that received the weakest supplemental light for a 24-hour period. Slowest and least vegetative growth occurred with an 18-hour photoperiod with the brightest supplemental light. The length of each axis was increased by decreasing the supplemental light. The length of the central axis was not altered by the photoperiod, but the length of the lateral axes increased as the photoperiod increased.

The average size of leaves increased as light intensity or photoperiod decreased.

Good tuber development took place with all treatments. In tuber production differences between treatments were not significant but variety differences were relatively great.

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ORIGIN OF THE SUBERIZED SEMIPERMEABLE MEMBRANE IN THE CARYOPSIS OF MAIZE [1]

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INTRODUCTION

The suberized semipermeable membrane in the caryopsis of the Gramineae has for the most part been considered to be derived from the integuments rather than from the nucellus. There has been little agreement or detailed evidence offered, however, in regard to the origin of the membrane, which lies immediately within the pericarp (pl. 1, *A–C*) in the mature kernel of corn (*Zea mays* L.). In some of the past histological work, even the presence of this membrane has not been mentioned. This lack of uniformity, in all probability, results from differences in technique employed by the various workers. Suberin and cutin have so little affinity for the histological stains ordinarily used that in the early stages in the development of the caryopsis the membrane, which is then very thin, is not easily identified by color differences. In sections of the mature kernel stained with Delafield's haematoxylin or Flemming's triple stain, the suberized membrane is seen as a hyaline line between the pericarp and aleurone layer (pl. 1, *C*), but at that stage in the development of the kernel it is much too late to determine the origin of the membrane. Earlier, there is a period in the development of the caryopsis when the membrane might appear to be a nucellar structure. This occurs after the disintegration of the integument and before the collapse of the cells of the epidermis of the nucellus. At that time the membrane adheres to and follows the contour of the outer surface of the nucellus (pl. 1, *D*). This stage also is too late to be useful in determining the origin of the membrane. Only when both the inner integument and the epidermis of the nucellus are present and separate is it possible to discover the original location of the primary membrane with any degree of certainty.

In some of the more recent work of this nature, staining difficulties have been avoided by the use of microchemical methods. By means of Sudan III or Sudan IV thin layers of suberin or cutin that otherwise might be overlooked can be demonstrated in the young caryopsis (pl. 2, *A, B*). Further indication of the nature and occurrence of the membrane may be obtained by treatment of the sections with sulfuric acid, in which suberin and cutin are insoluble (pl. 2, *C*).

The object of the investigation reported herein was to determine whether suberization of integumental origin occurs in the corn kernel.

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[2] The writer acknowledges her indebtedness to Eugene H. Herrling, of the Wisconsin Agricultural Experiment Station, for all the photography involved in the problem. Thanks are given also to Dr. J. G. Dickson and Dr. Emma L. Fisk for helpful criticism of the manuscript.

REVIEW OF LITERATURE

A review of recent papers dealing with the histology of the caryopses of various Gramineae in which microchemical staining methods were used indicates that suberization of the surface of one or both of the integuments is of common occurrence as compared with suberization of the nucellus.

Andersen (*1*),[3] using microchemical methods "suggested by Eckerson, Tunmann, and Molisch" in her study of *Poa pratensis* and *P. compressa*, after following the development of the caryopsis, stated (*1, p. 1014*) that in the mature caryopsis—

> the cell walls of the inner integument adjacent to the nucellus form a comparatively thin layer of suberin. The two rows of cells of the inner integument have collapsed somewhat, forming a narrow layer against the suberized layer of the outer integument. A layer of suberin is all that remains of the outer integument. The suberized layers of the inner and outer integuments are so tightly compressed that their identity can be seen only where the seed coat is torn in sectioning.

Eckerson, working with Harrington and Crocker (*5*), found suberin in the walls of the inner integument in both Johnson grass and Sudan grass, and the authors stated (*5, p. 219*) that—

> the coverings of the naked mature caryopses of both grasses consist of the fused pericarp and the inner integument, the outer integument and nucellus having entirely disappeared, with the possible exception of a portion of the latter over the micropyle.

In sections of the wheat kernel stained with Delafield's haematoxylin or Flemming's triple stain, Pugh, Johann, and Dickson (*9*) found a thin colorless membrane on the inner side of the inner integument and one of considerable and variable thickness on the outer side. The latter was thin over the embryo. They stated (*9, p. 625*):

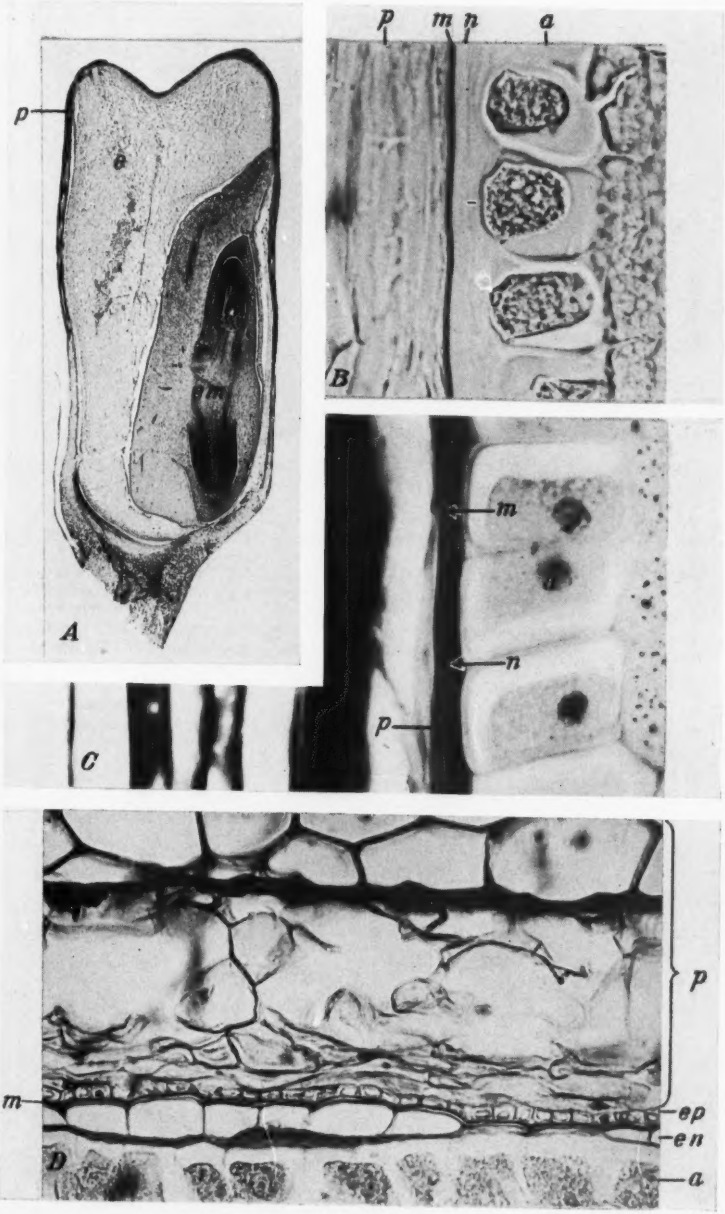
> Both membranes stain with Sudan III and are relatively resistant to sulfuric acid, indicating the presence of suberinlike or cutinlike substances.

Using Sudan III or Sudan IV on sections of barley kernels, Tharp (*13*) found that at flowering time, when as yet there was no structural contact between the integument and nucellus, there was a very faint trace of cutin on the inner and outer epidermis of the outer integument and a distinct cutin membrane on both surfaces of the inner integument, but none at that stage on the epidermis of the nucellus. By the time the embryo had reached the four-cell stage, the nucellar epidermis was adherent to the inner integument and the cutin membrane on the inner surface of the inner integument seemingly was common to both integument and nucellus. In the mature barley kernel the inner integument had a thin inner cutin membrane and a much thicker outer cutin layer of variable thickness, being very thin over the embryo, where it constituted an area of decreased resistance to permeation.

In the maize kernel also, Tharp found that the portion of the seed coat covering the embryo was more easily permeable to normal trichloracetic acid than the part covering the endosperm and that there were differences in the degree of permeation among varieties of corn.

Orton (*8*), in an earlier study of the permeability of seed coats of sweet and dent corn to mercury compounds, found that the rate of permeability to each of the four mercury compounds used also varied with the corn varieties.

[3] Italic numbers in parentheses refer to Literature Cited, p. 282.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EXPLANATORY LEGEND FOR PLALE 1.

A, Photomicrograph of a longitudinal section of a kernel of inbred line L harvested September 15, 1931, 42 days after pollination. Paraffin section stained with Flemming's triple stain. × 8.

B, Portion of a section near the crown of the kernel shown in *A*, showing position and thickness of the suberized membrane. Section stained with Sudan IV, mounted in Karo. × 600.

C, Portion of section shown in *A*, midway of the kernel. In this section, stained with Flemming's triple stain, the suberized membrane appears as a hyaline line. × 600.

D, Portion of a cross section of an immature kernel of strain A1237, open-pollinated, fixed September 6, 1930. Stained with triple stain. The integuments have practically disappeared and the hyaline membrane follows the contour of the remaining cells of the epidermis of the nucellus. × 440.

p, Pericarp; *e*, endosperm; *em*, embryo; *m*, suberized membrane; *a*, aleurone cells; *ep*, inner epidermis of the pericarp; *en*, epidermis of the nucellus; *n*, crushed remains of nucellus.

EXPLANATORY LEGEND FOR PLATE 2.

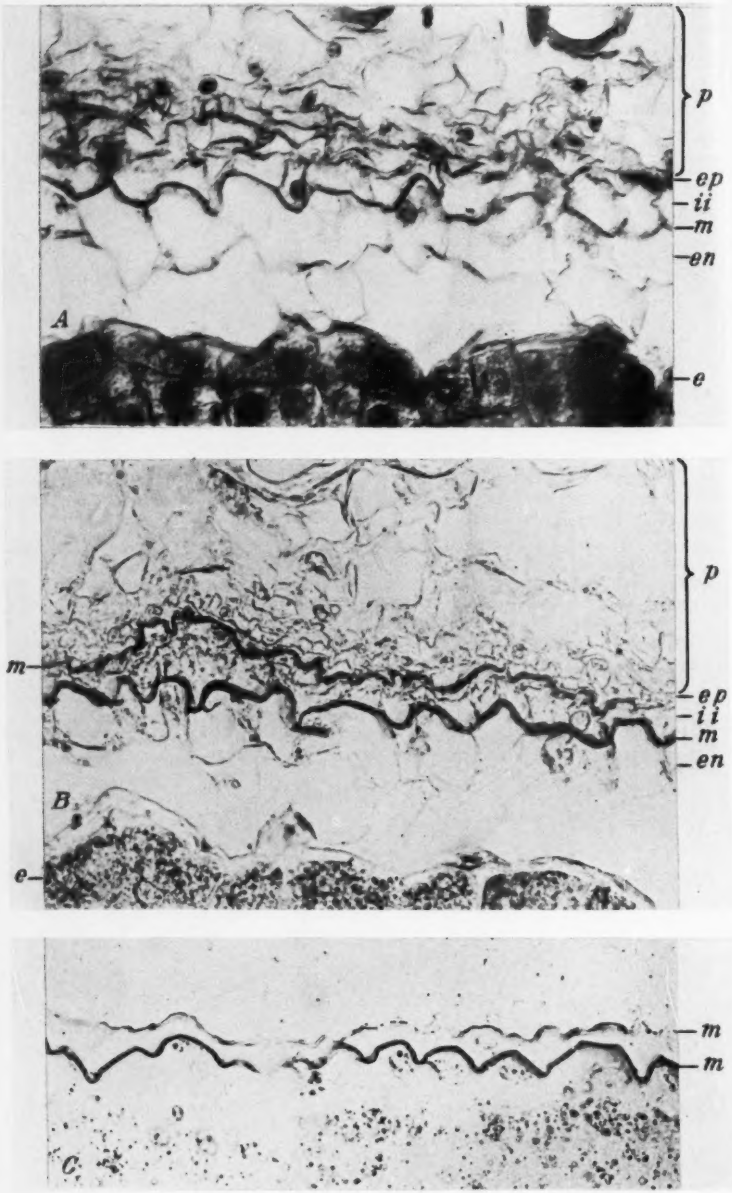
Identification of suberized membranes. Portions from approximately the same position in three sections of a developing caryopsis of inbred A48 fixed August 29, 1930, 15 days after pollination. × 480.

A, Stained with triple stain. The membrane is not easily identified by color differences at this stage of development.

B, Stained with Sudan III, mounted in glycerin. Membranes on both surfaces of the inner integument are easily identified. It should be noted that photomicrographs of sections of young caryopses sometimes give the impression of greater thickness in the red-stained membrane than is actually the case, because of a more or less three-dimensional view, due to the constant change of direction of the membrane and the depth of the section.

C, Stained with Sudan III and treated with sulfuric acid. All cellular structure has disappeared; only the suberized membranes retain their identity.

p, Pericarp; *ep*, inner epidermis of pericarp; *ii*, inner integument; *m*, suberized membrane; *en* epidermis of nucellus; *e*, endosperm.



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Beeskow [4] stated that the testa of the corn kernel is the layer that is directly responsible for the exclusion of nonpermeating substances. He found fatty and lipoid materials present and concluded that "the semipermeable character of the seed coat is tied up with a lipoid layer and a peculiar granular condition."

Johann (*6*), working with paraffin sections of young caryopses of corn, pictured a thin suberized membrane stained with Sudan III along the inner surface of the inner integument in contact with the nucellus, and observed (*6, p. 866*) that—

> attempts to demonstrate the presence of suberin on either of the adjacent walls when the epidermis of the nucellus and the inner integument were separable were unsuccessful. Thus, the origin of the membrane may be open to question. It seems probable, however, that the suberized layer in the corn kernel is derived from the integument, as is the case in wheat * * *, blue grass * * *, and Johnson grass * * *, although the number and position of the layers vary with the different genera.

Randolph (*11, p. 912*) outlined the course of development of the caryopsis of corn as follows:

> The major morphological changes in the transformation of the pistil into the mature caryopsis involve (1) the complete displacement of the ovule and integument tissues by the embryo and endosperm, (2) the formation of a suberized membrane derived from the epidermis of the nucellus, and (3) the transformation of the ovary wall into the pericarp.

He also stated (*11, p. 913*):

> A suberized nucellar membrane derived from the outer wall of the nucellar epidermis was present in the mature caryopsis as a continuous, well-defined layer between the aleurone and the pericarp.

In describing the methods used, Randolph stated that paraffin sections were stained either with Flemming's triple stain or Delafield's haematoxylin, and added (*11, p. 883*) that—

> freehand sections of fresh and fixed material also were utilized, and in making determinations of the composition of the cell wall Sudan IV and certain other stains were employed.

However, the photomicrographs presented show only sections stained with Delafield's haematoxylin.[5]

The methods by which the earlier workers arrived at their conclusions were not always stated in detail. None of the following authors cited has indicated that stains specific for suberin or cutin were used.

True (*14*), in 1893, studied the development of the caryopsis in corn, wheat, and oats. He noted the strongly cutinized outer walls of the nucellar epidermis of the corn kernel, adding (*14, p. 217*), "This cuticle is conspicuous in all stages of growth by reason of its great affinity for staining fluids." On the other hand, in speaking of the outer layer of the inner integument of the wheat kernel, he observed (*14, p. 222*) that "the hyaline outer layer is not easily demonstrated * * *."

Guérin (*3*), in 1899, described the development of the caryopsis in a number of the Gramineae. He may have seen the membrane in

[4] BEESKOW, H. C. THE SELECTIVE SEMIPERMEABILITY OF THE SEED COAT OF CORN. 1924. [Unpublished master's thesis, Univ. Chicago.]

[5] Information received in a letter from Dr. Randolph.

the corn kernel, though his report is not clear on this point and sheds little light on the origin of the membrane. He stated (*3, p. 7*):

> Du nucelle il ne persiste bientôt plus que l'épiderme, et le tégument externe de l'ovule disparaît totalement. Un peu plus tard, le tégument interne subit le même sort.
>
> * * * * * * *
>
> Quant au tégument séminal, il n'est représenté que par une bande comprimée dans laquelle il est impossible de retrouver la moindre structure cellulaire.
> L'épiderme du nucelle a de même complètement disparu.

Weatherwax (*15, p. 150*) considered that "the protective covering of the grain of corn consists of the *testa* and the *pericarp*. The former is the remnant of the integuments of the ovule * * *." In a later paper (*16*) he states that the nucellus is destroyed and none of it remains at maturity.

Randolph (*10*) studied sections of corn kernels stained with Delafield's haematoxylin and failed to note the membrane. She stated (*10, p. 6*):

> The absorption of the tissue in question was followed in a close series of stages, and in all the mature kernels studied there was in most cases no nucellar or integument tissue, the aleurone layer of the endosperm lying in close contact with the inner epidermis of the pericarp. In some scattered places a little unabsorbed material was seen, but it never formed a definite layer.

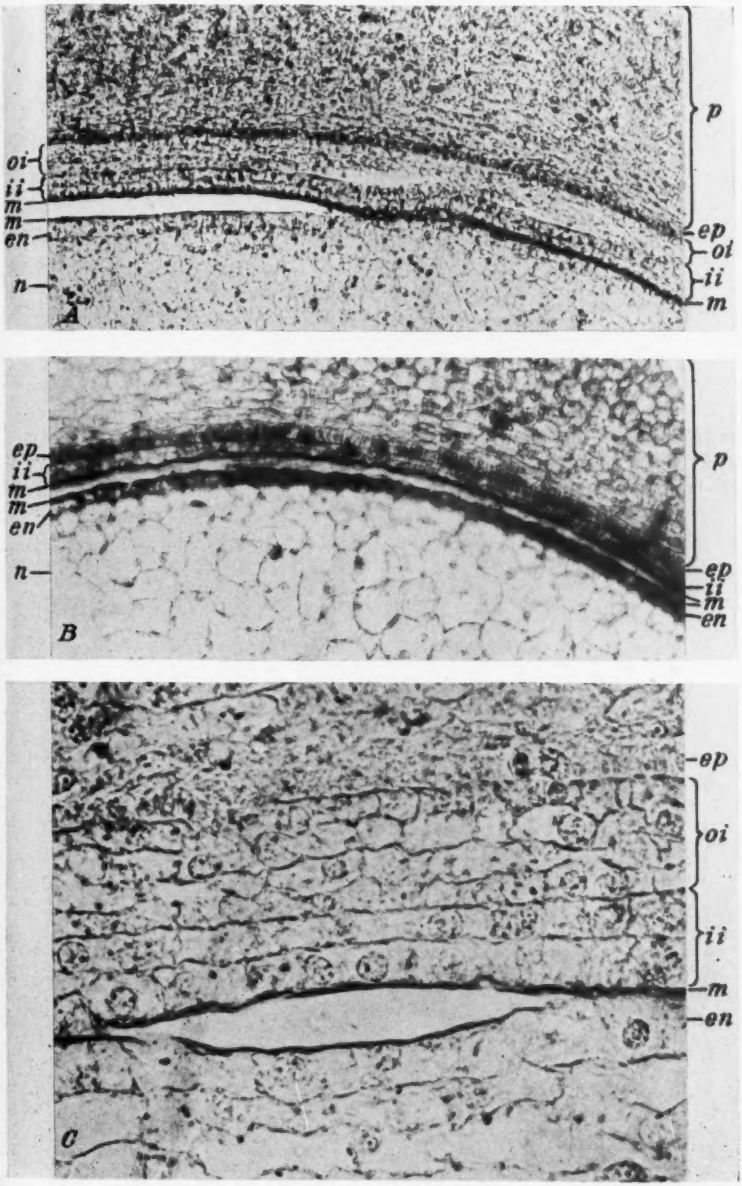
Haddad (*4*), also using Delafield's haematoxylin, listed the resorption of the tissues in the sweet corn kernel in the following order: Outer integument, inner integument, epidermis of nucellus, and the inner half of the ovary wall. He, also, did not recognize the presence of a suberized membrane.

MATERIAL AND METHODS

The yellow dent corn upon which this work is based was grown at Madison, Wis., in the summers of 1930, 1931, 1938, 1939, and 1940. The inbred lines used in the early years were the Illinois lines L, A48, A1237, and BR10; in 1938 and 1939, A48, Wis. 6, Wis. 26, Wis. R3, Ind. B2, and Ohio 56; and in 1940, a 5-year inbred from Funk's 176A, and Lan, a 7-year inbred from Lancaster Surecrop. Some ears were hand-pollinated, some were open-pollinated. Collections were made at intervals from 2 to several days during the early development of the caryopsis. Of the killing solutions used, chromacetic and a modification of Nawaschin's fluid were generally satisfactory. The specimens were embedded in paraffin by the alcohol-chloroform method.

Sections of the embedded material were stained with Flemming's triple stain or Delafield's haematoxylin to show the cellular structure of the caryopsis, and with Sudan III or Sudan IV to identify suberized or cutinized layers. Some of the latter sections were also treated with sulfuric acid as a further means of demonstrating the presence of suberin or cutin (pl. 2, *A–C*).

No attempt was made to determine the chemical nature of the membrane beyond the tests mentioned above, to which both suberin and cutin give a positive response. Eames and MacDaniels (*2*) stated that a thin cuticle is present sometimes even on the inner epidermis of the ovary and on the integuments of the ovules of vascular plants and added (*2, p. 36*), "In origin the cuticle is probably



FOR EXPLANATORY LEGEND SEE NEXT PAGE.

EXPLANATARY LEGEND FOR PLATE 3

Suberization of the inner integument and nucellus.

A, Freehand section of a young caryopsis of 176A, open-pollinated, harvested August 6, 1940. All silks were still green. The inner integument and nucellus, separated for a short distance, show membranes on both surfaces, the one on the inner integument being the more highly suberized. Stained with Sudan IV, mounted in glycerin. × 160.

B, Membrane shown on the inner surface of the inner integument on the germinal side of a young caryopsis in a freehand section of inbred Wis. 6, open-pollinated, harvested August 1939. A slight change in focus would have shown the presence of a thin membrane on the surface of the nucellus also. × 150.

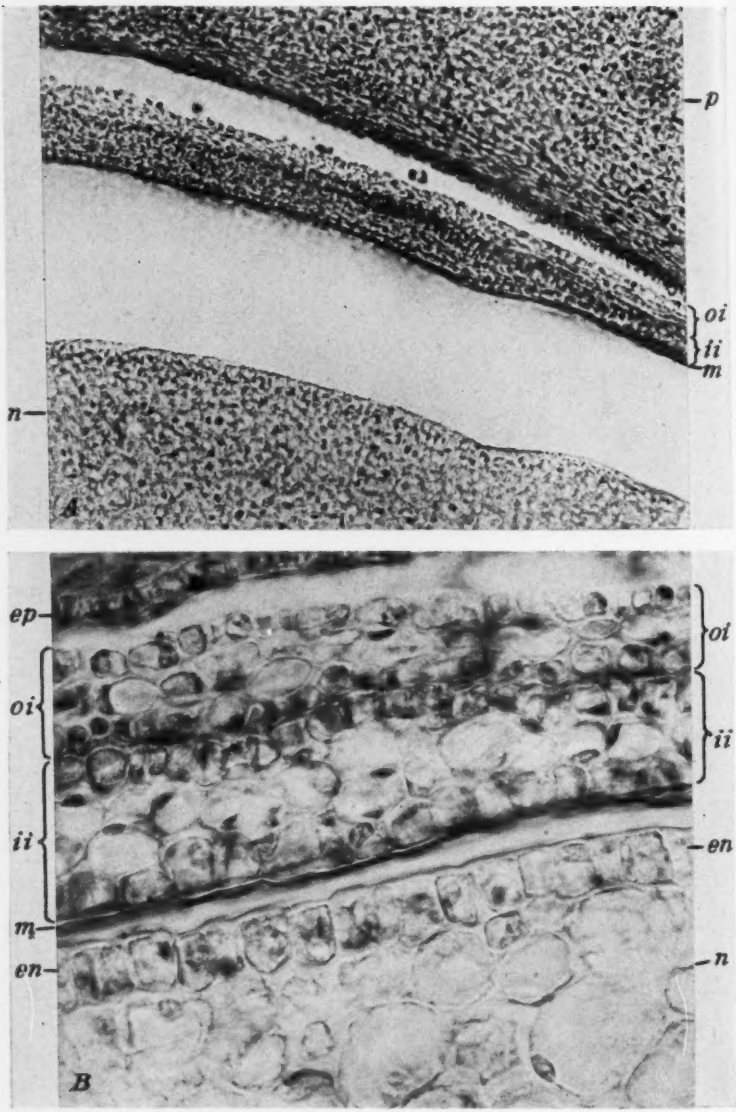
C, Suberin layer on the surface of the inner integument and of the nucellus where they have been separated. Paraffin section of young caryopsis of inbred A48 fixed August 17, 1938. Stained with Sudan III, mounted in glycerin. × 725. In all the sections (*A–C*) the cells of the inner integument appear to be normal and functioning.

p, Pericarp; *ep*, inner epidermis of pericarp; *oi*, outer integument; *ii*, inner integument; *m*, suberized membrane; *en*, epidermis of nucellus; *n*, nucellus.

EXPLANATARY LEGEND FOR PLATE 4

Freehand sections from two young caryopses of inbred 176A, open-pollinated, harvested August 6, 1940; stained with Sudan IV, mounted in Karo. A suberized membrane is present on the inner surface of the inner integument in both sections with none on the surface of the nucellus. *A*, × 160; *B*, × 700.

p, Pericarp; *oi*, outer integument; *ii*, inner integument; *m*, suberized membrane; *n*, nucellus; *ep*, inner epidermis of pericarp; *en*, epidermis of nucellus.



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a secretion of the protoplast of the epidermal cells * * *." Tharp (*13*) used the terms "cuticle" and "cutin" in relation to the membranes in barley kernels. On the other hand, Andersen (*1*) and Eckerson (see *5*) each noted suberized layers in the caryopses of the grasses that they studied.

In 1939 and 1940 fresh material was sectioned, stained with Sudan III or Sudan IV, and mounted in glycerin or Karo (*7*). These free-hand sections, cut without the aid of a microtome, supplemented the embedded material and, despite their obvious shortcomings, presented several advantages. They could be examined on the day of collection; separation of the nucellus and inner integument was shown more often; and the integuments were usually in a better condition than in the paraffin sections. Early stages in the formation of suberin were also more easily demonstrated, for apparently the fatty material deposited along the cell wall and not yet consolidated into suberin [6] had been removed from the paraffin sections to some extent by the chloroform used in the embedding process.

With the exception of plate 1, *A–C*, the figures presented are photomicrographs of cross sections of caryopses in early stages of development. Cross sections were preferred to longitudinal sections because in the former the cells of the inner epidermis of the pericarp presented a greater contrast in size and shape to those of the integuments and thus more sharply defined the outer limits of the integuments.

THE SUBERIZED SEMIPERMEABLE MEMBRANE

Sections of young caryopses of maize, fixed a week or more after pollination and stained with Sudan III or Sudan IV, usually show what appears to be a single thin red membrane between the inner integument and the epidermis of the nucellus, uniting the two surfaces and seeming to be common to both. From such sections it is difficult to determine the origin of the membrane. A little earlier, however, it is possible to find sections showing suberization in cases where the nucellus and inner integument are separated for longer or shorter distances. These sections indicate that suberization of both surfaces occurs (pl. 3, *A–C*), the primary membrane being formed as a thin layer on the inner surface of the inner integument well before degeneration of the adjacent cells of the inner integument has taken place and before suberin is found on the surface of the nucellus (pl. 4, *A*, *B*).

Degeneration of the inner integument begins on the germinal side of the young caryopsis. In cross sections of a developing kernel fixed 6 days after pollination, complete collapse of the inner integument for approximately 200μ on the germinal face in the region of the developing endosperm is shown (pl. 5). For the same distance the suberized membrane is very thin. Beyond that region the inner row of cells of the inner integument appears to be intact and functioning (pl. 5, *A* and *B*). As shown in plate 5, *C*, the membrane is thicker and more highly suberized in the region where the adjacent cells of the integument still persist. Such sharply marked differences

[6] Rhodes (*12*, *pp. 462–463*), after his study of the chemical nature of the suberin membrane in potato, concluded that "the suberin lamella of the cork cell arises by changes taking place in the fatty material resulting in the appearance of bodies no longer soluble in fat solvents.

"A relatively constant proportion of the soluble fatty substances never undergoes this change and the soluble component of the suberin lamella is mainly responsible for its staining properties."

as these in the integument are not evident in the epidermis of the nucellus, nor does this difference in thickness of the membrane occur in sections nearer the apex of the caryopsis, where as yet there has been no collapse of the inner row of cells of the inner integument.

Further evidence of the integumental origin of the primary membrane is seen in sections of young caryopses in which folding of the inner integument has occurred. Here the membrane follows the inner surface of the integument and in places is far removed from any contact with the nucellus (pl. 6, *A–D*). Folding of the inner integument was most pronounced in inbred 176A but was seen also in Lan and A48.

Later and more variable is the appearance of suberin on the outer surface of the inner integument in the abgerminal region and along the sides of the developing kernel. No instance of a complete coverage of the outer surface has been observed. The patches are thin, and vary in size and location (pls. 2, *B, C*; 7, *A*). They may be present in some strains of corn and absent in others. No suberization of the outer integument was observed in the specimens examined.

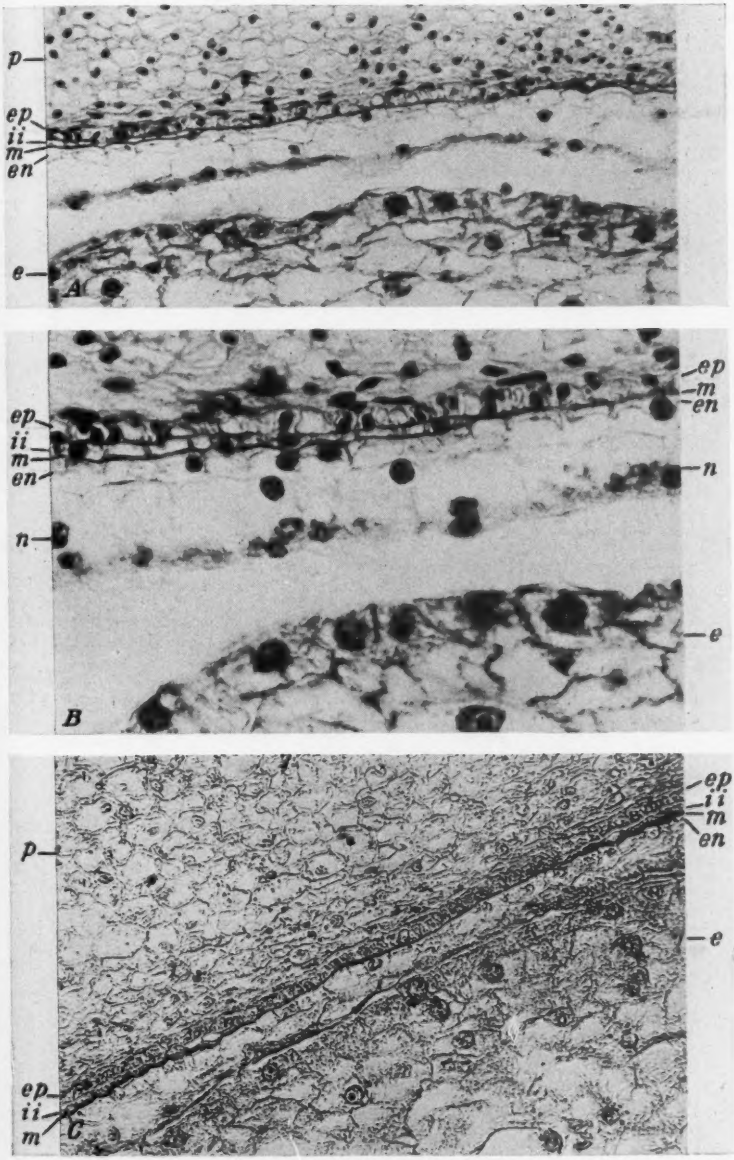
DISCUSSION

Changes in the developing caryopsis are rapid in the days immediately following fertilization. Randolph (*11*) has outlined in some detail the sequence of events in the degeneration of the inner integument in his material. His description applies in general to any of the inbreds used in the present study, but it does not take into account the suberization of either the inner or outer surface of the inner integument at any stage of development. He considers the suberized semipermeable membrane to be entirely a nucellar structure, and states (*11, p. 908*), "Disintegration of both integuments is well advanced before the nucellar epidermis becomes extensively suberized." The material examined in the present study fails to support this view.

Although degeneration usually begins in the inner integument earlier than in the epidermis of the nucellus, reference to plates 3 to 7 shows that no valid argument can be raised against the integumental origin of the suberized membrane if it is based on the inability of the inner integument to produce such a layer because of the prior degeneration and resorption of its cells.

A membrane is shown to be present on the inner integument earlier than on the nucellus (pl. 4). For this reason and because of its greater thickness during the time the integument and nucellus may be separated it is considered to be the primary membrane. After the two tissues have become physically united it is difficult to determine the subsequent contribution of fatty material made by each toward increased thickness of the membrane, which remains thin at best.

Variations in the thickness and permeability of the membrane in different areas in the mature kernel correspond with changes that have taken place in the integument. In the mature kernel the membrane is most permeable in the embryo region, the region where degeneration of the integument began (pl. 5). It is less so on the sides and in the abgerminal area, where the integuments persisted for a longer time and where the primary membrane is probably reinforced,



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EXPLANATORY LEGEND FOR PLATE 5.

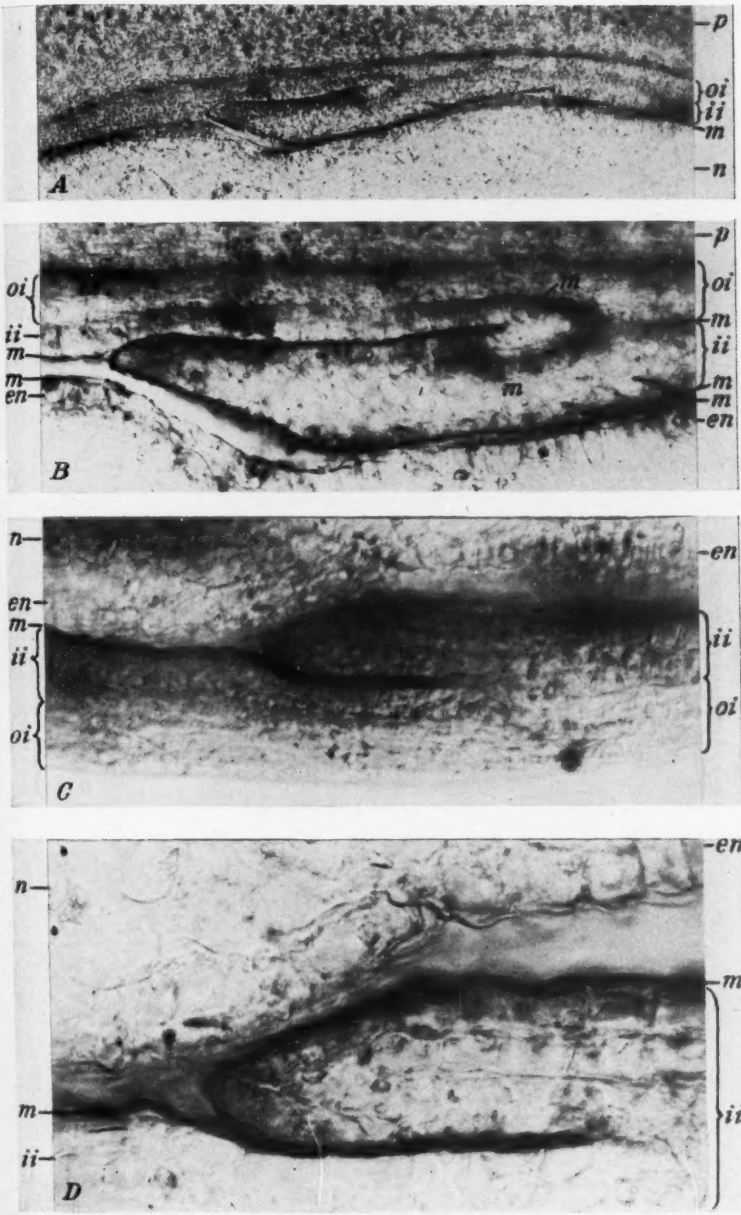
Portions of paraffin sections of inbred A48 fixed August 23, 1938, 6 days after pollination, showing the membrane to be thin in the germinal region where the inner integument has collapsed.

A, In the center of the section complete collapse of the inner integument has occurred; on each side one row of cells of the integument remains. Stained with triple stain. × 240.

B, Detail of the section next in the series to the one in *A*, showing nuclei in remaining cells of the inner integument. × 460.

Section from the next slide of the series shown in *A* and *B*, stained with Sudan III, mounted in glycerin. The membrane is more highly suberized in the region where the inner row of cells of the inner integument still persist than in the area where complete disintegration of the integument has occurred. × 240.

Pericarp; *ep*, inner epidermis of pericarp; *ii*, inner integument; *m*, suberized membrane; *en*, epidermis of nucellus; *n*, remains of nucellus; *e*, developing endosperm.



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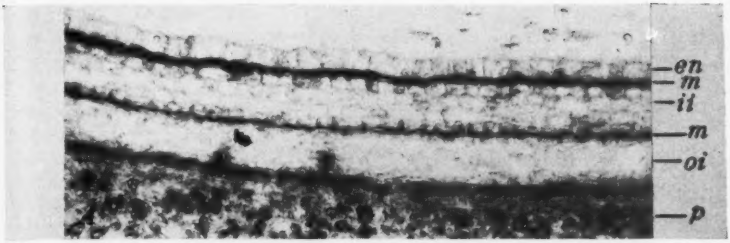
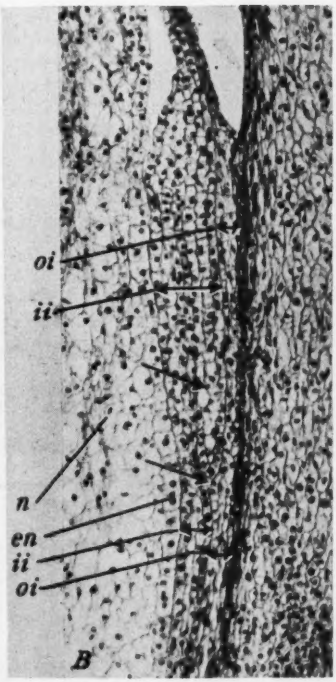
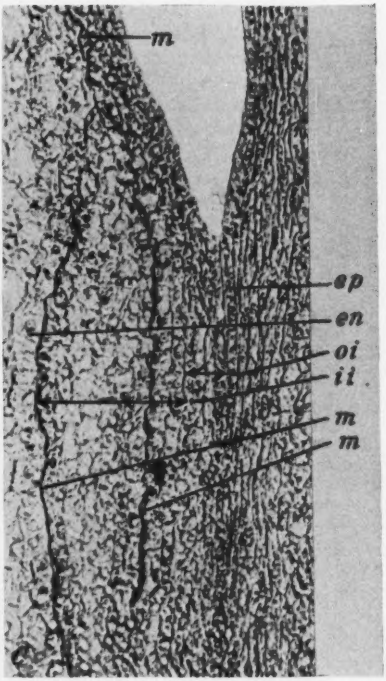
EXPLANATORY LEGEND FOR PLATE 6.

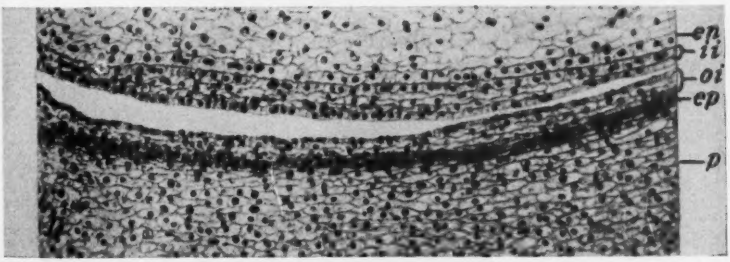
Folding of the inner integument along the sides of two young caryopses of inbred 176A. Freehand sections stained with Sudan IV, mounted in glycerin. A suberized membrane is present on the inner surface of the folded inner integument. *A* and *B*, Different magnifications of the same section; *B* shows the surface of the inner integument to be more highly suberized than the surface of the nucellus. Slight suberization of the outer surface of the inner integument near the base of the fold is also shown. *A*, × 85; *B*, × 210. *C* and *D*, Different magnifications of a section from another young caryopsis. A suberized membrane is present on the inner surface of the inner integument, none on the surface of the nucellus. In the folds observed, the apex pointed toward the germinal face of the caryopsis; the backward bend of the inner integument was toward the outer integument rather than toward the nucellus. *C*, × 280; *D*, × 590.

p, Pericarp; *oi*, outer integument; *ii*, inner integument; *m*, suberized membrane; *n*, nucellus; *en*, epidermis of nucellus.









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EXPLANATORY LEGEND FOR PLATE 7.

A, Portion of a freehand section from the abgerminal region in a young caryopsis of inbred R3 harvested in August 1939, showing membranes stained with Sudan III on both surfaces of the inner integument. × 200 approximately.

B, A fold in the inner integument in a paraffin section of a young caryopsis of 176A stained with triple stain. No distinguishing color differences were obtained in the cell walls. The lower arrow points to the turn in the outer layer of cells of the inner integument at the base of the fold. The upper arrow indicates the approximate position of the bend of the inner epidermal cells of the inner integument, although the turn of that layer is not clearly shown in this section. × 145.

C, Another section through same fold as *B*, stained with Sudan IV, showing presence of a membrane outlining the fold. × 280.

D, Thickening, without folding, of the inner integument along the side of a young caryopsis of A48, fixed in 1938. The relatively thick appearance of the wall between the epidermis of the nucellus and inner integument in *B* and *D* may be accounted for largely by the fact that it represents the combined outer epidermal walls of two tissues. × 140.

en, Epidermis of nucellus; *m*, suberized membrane; *n*, nucellus; *ii*, inner integument; *oi*, outer integument; *p*, pericarp; *ep*, inner epidermis of pericarp.

after the disintegration of the intervening cells, by whatever thin patches of suberin may have been laid down on the outer surface of the inner integument (pls. 2, *B*, *C*; 7, *A*).

In those strains of corn in which folding of the inner integument has occurred, evidence of the integumental origin of a suberized layer appears to be conclusive. The membrane adheres to the inner surface of the inner integument, following its folds irrespective of their position with regard to the nucellus (pls. 6, *A–D*; 7, *B*, *C*).

The fact that pronounced folding of the inner integument occurs in some strains of corn has received little if any mention in studies of this problem. The probable explanation of its occurrence seems simple. The integuments are delicate structures, for the most part 2 to 4 cells thick, attached at the base of the ovule but otherwise distinct for a time in the narrow space between the spherical ovule and the concave inner surface of the ovary wall. The inner integument completely surrounds the ovule except at the micropyle; the outer integument does not completely cover the inner integument. During its development the inner integument must adjust itself to the curved space in which it is confined. If its growth is considerably more rapid or if it is continued longer than that of the neighboring parts, folding would seem inevitable.

Although such increased growth of the inner integument as is indicated by thickenings (pl. 7, *D*) or folds of various depths [7] may not be present in all cases, there seems to be no reason to believe that the origin of the primary membrane is essentially different in different strains of corn. Randolph studied varieties of dent corn as well as sugary and flint types, and stated (*11*, *p. 882*) that "no significant morphological differences were noted among the different types other than variations which might readily be attributed to differences in size, shape, or degree of maturity of the kernels."

SUMMARY

In the caryopsis of maize thin layers of suberin are formed on the surface of both the inner integument and the epidermis of the nucellus, the primary membrane being formed on the inner surface of the inner integument. The two membranes become physically united early in the development of the caryopsis and thereafter appear to be a single membrane common to both surfaces, much as is the case in barley. The membrane is thinner and more permeable on the germinal face of the kernel, a region where complete collapse of the cells of the inner integument first occurs.

In some strains of corn, areas on the outer surface of the inner integument also become lightly suberized. No instance of a complete coverage of the outer surface of the inner integument was observed, and no suberization of the outer integument was seen.

In young kernels in which folding of the inner integument occurs, the primary membrane is shown to adhere to the folded inner surface of the inner integument regardless of its position in relation to the nucellus.

[7] Guérin (*3*, *p. 55*) may have observed a somewhat similar condition when he stated:
"L'ovule est toujours bitégumenté, et chaque tégument ne comporte, d'une façon générale, que deux assises cellulaires seulement. Parmi les genres que nous avons examinés, les *Zea*, *Tripsicum*, *Coix*, du groupe des Maydées, font exception à cette règle. Nous avons vu, en effet, que les téguments ovulaires peuvent, dans ces genres, comporter ensemble six et huit assises de cellules."

It appears, therefore, that maize conforms to the general pattern of the Gramineae in which the suberized membrane (or membranes) within the caryopsis is derived from one or both integuments. From the material examined in the present work it is concluded that the suberized semipermeable membrane in the corn kernel is derived primarily from the inner integument.

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APHID INFESTATION OF STRAINS OF CORN AS AN INDEX OF THEIR SUSCEPTIBILITY TO CORN BORER ATTACK [1]

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INTRODUCTION

The European corn borer (*Pyrausta nubilalis* (Hubn.)), a chewing insect, and the corn leaf aphid (*Aphis maidis* Fitch), a sucking insect, are both pests of corn (*Zea mays* L.). Differential susceptibility among corn strains to the corn borer has been recognized for several years. For corn-growing areas where the borer is a serious pest, the differential susceptibility to the insect among hybrid strains of corn provides a basis of major importance in the classification of breeding material. It is now known that there are similar differentials in susceptibility to the corn leaf aphid. The purpose of this paper is twofold: (1) To present data showing relations between the susceptibility of corn hybrids to aphid and to borer injury and, on the basis of these relations, (2) to suggest a new technique for evaluating susceptibility of hybrids to corn borer injury.

Burdensome techniques constitute the chief difficulty in evaluating susceptibility of corn strains to injury by larvae of the borer. The problem of sampling is a difficult one. In general, the choice has been between the difficult procedure of obtaining adequate samples of the stalks of the different strains under natural field infestation or of stalks that have been manually infested, and that of feeding etiolated leaves of the different strains to young larvae under laboratory conditions. Manual infestation reduces the number of plants needed for dissection but adds the requirements of rearing moths and applying their eggs to the strains under test. Further, the data from stalk dissections are subject to a large error unless the dissections are properly timed with reference to the usually different maturity of the strains under study. Laboratory feeding experiments also require the rearing of moths and the handling of eggs and larvae in addition to the necessity of providing a constantly fresh supply of food. Both methods may add an uncertainty of simulating natural conditions. A third choice, less often used, but satisfactory when populations are high and damage severe, has been to rate quantitatively the degree of larval damage to the leaves.

Since the inbred strains from which the corn hybrids are made are weak and consequently slow growing, they seldom carry sufficient natural populations of the corn borer to permit reasonably accurate selection. A simple technique, therefore, which can be used more advantageously on the inbred lines as such is needed.

[1] Received for publication August 15, 1941. Cooperative investigations of the Departments of Entomology and Agronomy, Ohio Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

REVIEW OF LITERATURE

Methods of studying strain differences in borer populations have been reported by Huber, Neiswander, and Salter;[2] Meyers, Huber, Neiswander, Richey, and Stringfield;[3] and Patch.[4] Correlations between borer and aphid populations on corn have been pointed out by Huber and Stringfield.[5] In the determination of strain susceptibility, all these investigators have relied upon natural field infestation, manual infestation, or both. The last method has been used extensively by Patch in studying the behavior of the borer under a wide range of conditions. Since 1937, Huber (unpublished data) has employed laboratory methods in which newly hatched larvae are fed etiolated portions of corn leaves from different strains for a period of 2 or 3 weeks.

MATERIALS AND METHODS

The strains of corn utilized in this study were developed or introduced by the cooperative corn project in Ohio and included inbred lines, commercial and experimental hybrids made from these lines, and the open-pollinated variety Woodburn.

In general, the material studied was from the regular breeding nurseries and field performance experiments. The inbred lines were in single-row or 4-row plots about 35 feet long. The number of replications was variable, depending upon how widely a given line was being used for seed increase and crosses. The hybrids were principally in regular field performance tests variously located in the State, and planted in 2- by 10-hill plots in fivefold replication or in 2- by 15-hill plots in fourfold replication within a modified Latin square design.[6]

ESTIMATING APHID SUSCEPTIBILITY

The degree of aphid susceptibility was estimated either by counting the number of stalks with aphid colonies on the newly emerged tassels or by counting the number of stalks determined to be barren because of heavy aphid population. Aphids begin to be noticeable just as the tassels emerge. For a period of several days, depending somewhat on weather and food conditions, the aphids remain in aggregations. As the tassels lose their succulence, the aphid colonies break up, and the individual aphids find food and shelter on other parts of the plant or are lost. After such dispersal, counts of infested tassels are not dependable. Observation has shown, however, that plants severely infested with aphids are commonly barren, or nearly so; hence the percentage of barren plants was taken as an estimate of aphid injury in experiments where tassel infestation was not obtained and where it was obvious that aphids had been an important cause of barrenness. In some tests, both measures were used.

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APHID RATING

In these studies, it has been necessary to take data in such plantings as were found to be infested rather than in systematically planned experiments, since it could not be known in advance which plantings would carry heavy insect infestations. Such data generally lack an element of orderliness, because some strains will be represented more or less often than others and in different plantings. The problem of compiling the data was met as follows:

(1) Since the inbred line Indiana WF9 was both highly susceptible and common to all plantings where inbred lines were studied, it was assigned a value of 100. Each other inbred line was assigned a rating in each planting by expressing its percentage of infested plants as a percentage of the corresponding infestation of line Indiana WF9.

(2) The hybrid Ohio K35 was also both highly susceptible and common to many different plantings. Accordingly, this hybrid was assigned a value of 100 and all other hybrids included in this study were rated in comparison with Ohio K35.

This procedure of comparing all strains to one which is used as a standard has its obvious weaknesses. It tends to make data more variable by introducing the variability of the standard. If, however, sets of such data from independent sources show definite relations to each other, the inference would be that there is a real relation, although its measurement is probably less precise.

PRESENTATION OF DATA

Table 1 presents a compilation of the data both on the inbred lines and on their single crosses, with the purpose of establishing an aphid susceptibility rating for the various lines. It will be noted that the number of tests involved ran from 1 to 18 for each line evaluated. Of the total of 18 tests, 15 were single-cross and 3 were inbred-line tests. Where single crosses were used, the value was the average of all the infestation counts where the line in question was one of the parents. Depending upon the degree of dominance and the susceptibility of the other parent lines, the single-cross ratings cannot be wholly comparable but are recorded as representing the best data available at this time.

TABLE 1.—*Relative aphid ratings of 30 inbred lines of corn, with Indiana WF9 assigned a value of 100 in each comparsion, 1938–40*

Inbred line	Number of tests	Relative rating	Inbred line	Number of tests	Relative rating
Ohio 02	6	201	Ohio 28	6	57
Iowa Cl 447	2	180	Ohio 17	3	55
Ohio 84	1	152	U. S. 187–2	8	51
U. S. 4–8	6	121	Iowa L289	3	51
Indiana Tr	4	108	Illinois R4	4	50
U. S. 540	5	105	Indiana 38–11	5	49
Iowa Os426	3	101	Wisconsin CC5	2	34
Indiana WF9	18	100	Ohio 33	10	31
Iowa Os420	10	93	Ohio 51A	12	31
Ohio 26	10	93	Iowa I205	4	25
Illinois A	6	85	Ohio 07	2	25
Illinois 90	4	77	Ohio 40B	12	19
Ohio 56	3	74	Iowa L317	7	18
Ohio 65	4	72	Ohio 67	3	14
Illinois Hy	9	68	Ohio 51	4	9

Table 2 shows the predicted and observed ratings for aphid susceptibility of a group of double crosses included in the cooperative corn performance tests. The ratings of the four parent lines of each double cross are averaged and are given in the fourth column as the predicted rating for the double cross. The observed ratings in the fifth column represent the mean of the relative ratings of the aphid infestation on each planting with Ohio K35 used as a standard of comparison in each test. The correlation coefficient between the predicted and observed aphid ratings, as expressed by the values in table 2, is 0.53, a significant correlation.

TABLE 2.—*The predicted aphid rating of 28 double crosses on the basis of aphid ratings of parental inbred lines and the actual percentage of stalks of double-cross hybrids infested with aphids or that were barren because of aphids, in 16 cooperative test plantings, 1938–40*

Hybrid	Pedigree	Number of tests	Predicted rating	Actual mean aphid rating
Ohio 465	(56 × Os420) (65 × 02)	2	110	54
Ohio K35	(65 × 02) (Hy × 26)	16	109	100
Iowa 931	(L289 × C1 447) (Os 420 × Os426)	4	106	75
Ohio 38	(WF9 × Hy) (40B × 02)	6	97	109
Indiana 608B	(A × Tr) (WF9 × Hy)	2	90	122
Ohio W17	(56 × 4–8) (51 × 84)	16	89	74
Ohio 36	(51A × WF9) (40B × 02)	1	88	83
U. S. 44	(187–2 × 4–8) (Hy × 540)	3	86	88
Indiana 425	(A × 90) (WF9 × Hy)	3	83	119
Ohio K23	(26 × 51) (65 × 84)	7	82	54
Illinois 172	(R4 × Hy) (A × 540)	3	82	32
U. S. 52	(Hy × 67) (4–8 × 540)	5	77	118
Illinois 384	(A × Hy) (WF9 × R4)	5	76	56
U. S. 65	(51 × 4–8) (Hy × 540)	15	76	58
Ohio 563	(WF9 × Os420) (51A × Hy)	1	73	37
Ohio 387	(WF9 × 38–11) (51 × 4–8)	2	70	78
Ohio 160	(Os420 × Os426) (51 × 56)	1	69	6
Iowa 939	(L289 × I205) (Os420 × Os426)	16	68	68
Indiana 614	(A × Hy) (R4 × Tr)	2	68	37
Ohio 26	(WF9 × Os420) (33 × 40B)	3	61	97
Ohio M15	(51 × 26) (A × CC5)	2	55	40
Ohio 54	(WF9 × 40B) (51A × Hy)	3	55	52
Ohio 418	(40B × 4–8) (51 × 65)	4	55	10
Ohio 398	(51 × 4–8) (Hy × L317)	2	54	35
Ohio 487	(51 × 56) (Os420 × 40B)	1	49	0
Ohio 30	(WF9 × 07) (33 × 40B)	3	44	45
Ohio C14	(51 × 56) (67 × Hy)	9	41	54
Ohio 32	(28 × 187–2) (33 × 40B)	6	40	48

Table 3 gives the corn borer population for a group of hybrids grown at Holgate, Ohio, in 1936, on which the population counts were taken in July; and the expected aphid rating was taken from values in table 1. The correlation coefficient between the number of borers per stalk, column 2, and the estimated aphid rating, column 3, is 0.74, a significant value.

One of the experimental plantings for comparing the field performance of single crosses in 1939 was located at Van Wert, Ohio. The 100 strains included in this test ranged from an average of 1 to an average of 67 percent of the stalks infested with aphids, and from 0 to 18 percent of the stalks were barren and heavily infested with aphids. Corn borer populations varied from 2.5 to 6.8 borers per stalk. Additional data taken on these plots included counts of borer holes in unit areas of the stalk as a measure of borer population, midsilking date as a measure of relative maturity, and stalk height at the time of moth flight as a partial measure of egg deposition.

The partial correlation of aphid infestation and borer holes per stalk with height and silking taken into account was 0.29, a significant value.

TABLE 3.—*Borer population per stalk and estimated aphid rating for a group of single, three-way, and double crosses, Holgate, Ohio, 1936*

Hybrid	Pedigree	Borer population per stalk	Estimated aphid rating
Iowa 931	(L289 × C1 447) (Os420 × Os426)	2.2	106
Iowa 939	(L289 × I205) (Os420 × Os426)	2.2	68
	(540 × 67) × L317	1.7	46
	(65 × 51) (17 × 26)	1.7	57
	(56 × 67) × L317	1.5	35
	(Tr × 67) × L317	1.4	47
Ohio W17	(51 × 84) (56 × 4-8)	1.5	89
	(Hy × 67) × L317	.9	33
	(R4 × 67) × L317	.8	27
	(540 × Hy) (67 × L317)	.9	51
	(67 × L317)	.7	16

A similar comparison involving 70 strains, mostly single crosses, at Van Wert in 1940, carried a borer population averaging nearly 12 borers per stalk and an aphid infestation up to 24 percent. With this high borer population, strain differentials in leaf and stalk damage in late July were conspicuous and measurable. Because it was not possible to obtain the borer population for each individual strain, each strain was rated as to the relative amount of damage to the stalks and leaves. Strains with almost no leaf punctures or very few (fig. 1) were rated 0, whereas those showing holes and ragged leaf punctures (fig. 2) in practically every leaf, together with a majority of leaf midribs broken, were classified in the highest category, viz, 4. Strains with an intermediate amount of damage were placed in intermediate categories. Since there is a significant relation between such measurements and borer population and since leaf-damage measurement is more rapid, it was used as a measure of larval population. The correlation of this value with the percentage of stalks showing aphid infestation was 0.44, a significant value. The same measurement of leaf and stalk damage showed a correlation of 0.40 with the number of borer holes per stalk. This correlation is also significant.

On 19 strains, including single-cross, three-way, and double-cross hybrids planted at Van Wert in 1940, data were taken on the number of eggs and borers per stalk. The average egg masses per stalk on July 13 ranged from 1 to 2.4 on the different strains, and the borer population in early August, from 10.2 to 18.5 per stalk. Aphid infestation on these plots was negligible. However, the predicted aphid ratings of these strains showed a partial correlation of 0.83 with borer population when the number of egg masses per stalk was taken into account.

Additional evidence of the relationship between borer and aphid populations is shown in data taken from 33 inbred lines planted for comparison in fourfold replications of 4- by 10-hill plots at Van Wert in 1940. Egg counts were made and borer population was determined on only 16 of these 33 strains. The partial correlation of borer population and the percentage of stalks infested with aphids,

FIGURE 1.—Representative leaves of a corn strain rated in the 0 category of damage by corn borer larvae.

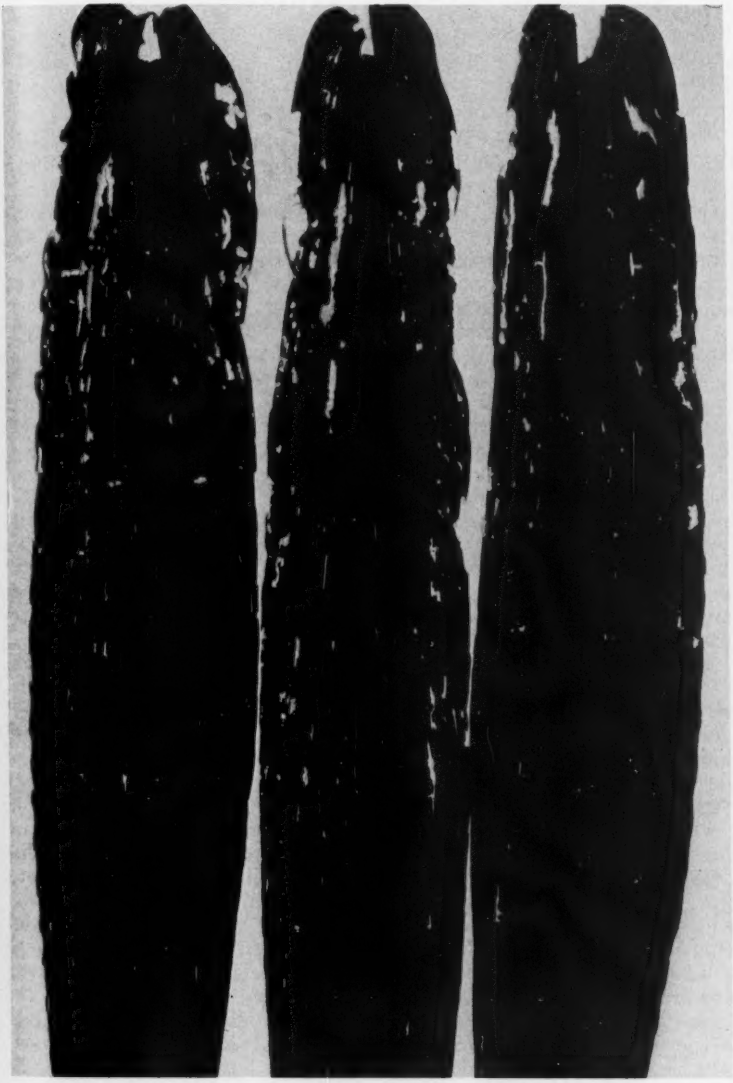

FIGURE 2.—Representative leaves of a corn strain rated in category 4 of damage by corn borer larvae.

with eggs taken into account, was 0.50, a significant correlation. When borer holes per stalk were used as a measure of borer population on the entire planting of 33 strains, the correlation with the percentage of aphid-infested stalks was 0.37. This also is a significant value.

DISCUSSION

The ratings on aphid susceptibility of inbred lines as indicated in table 1 are obviously subject to considerable error. As further data are accumulated, these values will fluctuate. For example, inbred line Ohio 84 is rated on the basis of one test only so far as its comparison with Indiana WF9 is concerned. The level of aphid infestation in the various comparisons involved ranged from a maximum of 9 percent in 1 planting to 95 percent in another, with an average of about 35 percent for the total of 18 plantings.

The ratings of the double crosses in table 2 are also subject to error. The total of 28 hybrids include 4 with only 1 test. Of these 4 hybrids, 1 is new and the other 3 were discarded as commercially undesirable after the first test. Where the percentage of stalks infested was used as the basis of aphid rating on the double crosses, the maximum infestation on 12 plantings ranged from 12.4 to 67.3 percent. On 4 of the double-cross tests where the percentage of barren stalks was used as a measure of aphids, the percentage barren ranged from 3.8 to 8.3. A further source of error in rating double crosses may be a considerable degree of dominance at either the high or low end of the susceptibility scale. Moreover, the ratings are based on data assembled from fields in various locations subjected to widely different environmental conditions, factors which in themselves are likely to introduce variability in the relative ratings.

With these considerations in mind, the correlation coefficient of 0.53 between the predicted and observed aphid ratings of the double crosses (columns 4 and 5, table 2) is interpreted as being reasonably high. It indicates that the relative susceptibility to aphid injury of inbred lines is transmitted to their hybrid progenies in a measurable degree, and that knowing the susceptibility of inbred lines, one can reasonably make predictions as to the susceptibility of their crosses.

The purpose of this paper, however, is not primarily to demonstrate the inheritance of susceptibility to aphids, but rather to use the facts involved to build hybrids less susceptible to the corn borer. The problem is to demonstrate the relation, if any, between the behavior of the aphid and the borer on the same strains of corn. Evidence of a positive relation is indicated in the partial correlation of 0.29 between borer holes per stalk with height and silking taken into account. Although this correlation is low, it is significant. In connection with this particular test, there was evidence of considerable competition between aphids and borers. Aphids began to draw heavily on the plant nutrients earlier than the borers. As the injured plant is starved, it differentiates more slowly and becomes the type of plant that has long been known to provide a poor medium for borer development.

Further evidence of a relation between the susceptibility of corn to aphid and to borer injury appears in the correlation of 0.44 between leaf damage as caused by the borers while still in the first to third instars and the percentage of aphid-infested stalks. The partial

correlations of 0.83 between actual borer populations per stalk and predicted aphids with egg masses taken into account, and of 0.50 between borer population and the percentage of aphid-infested stalks with eggs taken into account, together with the correlation of 0.74 between borer population and predicted aphids at Holgate, all indicate that strains preferred by the aphid are in general also preferred by the borer. Further evidence of this relation is the correlation of 0.37 between borer holes and the percentage of stalks infested with aphids. It is not within the province of this discussion to suggest the possible factors involved, but it does seem that the relationship is not only unique but also that it can be made to serve a useful purpose. It would appear that the information at hand can be used to build hybrids not only less susceptible to the aphid but at the same time less susceptible to the corn borer.

SUMMARY AND CONCLUSIONS

Data are presented to show (1) that inbred lines of corn and their hybrids exhibit heritable differences in susceptibility to the corn leaf aphid, and (2) that susceptibility to the corn leaf aphid, a sucking insect, was measurably correlated with susceptibility to the corn borer, a chewing insect. It would seem that at least preliminary classifications of breeding material in respect to susceptibility to the corn borer might be made on the basis of aphid populations. Aphid infestations can be estimated rapidly, whereas corn borer populations must be estimated by slow and burdensome methods.

445016—42——4

AN IMPROVED METHOD FOR DETERMINING THE DISTRIBUTION OF SALT AND WATER IN CURED HAMS [1]

By A. K. BESLEY, *associate bacteriologist, Animal Nutrition Division, Bureau of Animal Industry, United States Department of Agriculture*, and FLOYD CARROLL, *research assistant, Animal and Dairy Husbandry Department, University of Maryland*

INTRODUCTION

Of fundamental importance in the production of cured hams is a knowledge of the action of the curing ingredients within the meat. The composition of curing mixtures and their method of application to hams have been the subject of study by several investigators (*10, 13, 22*).[2] In most of this work, however, results have been expressed in terms of palatability and chemical analyses based on the entire ham and have contributed little information on the distribution, within the meat itself, of the curing ingredients. Other workers (*2, 16, 18*) have shown that different portions of cured hams vary in their content of curing ingredients, and that curing and aging procedures affect the distribution of salt in hams (*11, 17, 20, 24*).

Callow (*6, 7, 8*) has reported rates of salt and water absorption and dextrose penetration into pork muscle or parts of muscle. These papers, together with the works of Richardson (*23*) and Brooks (*5*) on the effect of nitrate and oxygen on the color of cured pork, have been reviewed and interpreted by Jones (*14*). However, these studies were conducted usually as in vitro experiments; consequently, there is no assurance that the results apply equally to meat within the entire ham. Variations in the composition of the different ham muscles, together with their relative location within the ham, may influence processing results. Table 1 gives determinations by the present authors of the ether-extract, water, and crude-protein content of the ham muscles arranged in the order of their ether-extract content. The importance of a knowledge of the quantity of fat and connective tissue in pork and their relation to the rate of salt penetration has recently been demonstrated by Banfield and Callow (*1*) as well as by Clarenburg (*9*).

Since salt [3] is universally used as the principal preservative in cured hams, the role played by this ingredient during the processing procedure is of particular importance. A ham, however, is not sufficiently homogeneous to permit accuracy in random sampling unless the material has been thoroughly ground. Grinding an entire ham, on the other hand, mixes its component parts, and samples from such material fail to provide information on the distribution of the curing ingredients throughout the ham. Consequently, in studies to determine the factors responsible for the rate of salt absorption, entire hams have usually been processed, and selected parts have served as the sources of sampling material.

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[1] Received for publication June 28, 1941.

[2] Italic numbers in parentheses refer to Literature cited, p. 303.

[3] The term "salt" as used throughout this manuscript refers to sodium chloride.

TABLE 1.—*Ether-extract, water, and crude-protein content of 10 muscles dissected from a 13-pound fresh ham*

Muscle	Ether extract	Water	Crude protein [1]
	Percent	*Percent*	*Percent*
Vastus lateralis	3.37	77.32	94.36
Adductor	4.47	75.02	92.94
Semimembranosus	5.34	74.13	92.35
Rectus femoris	5.66	75.51	92.13
Sartorius	6.54	74.00	91.77
Vastus medialis	7.05	73.70	93.25
Gracilis	7.10	72.29	92.35
Biceps femoris	7.11	74.36	94.73
Semitendinosus	8.50	73.38	92.35
Vastus intermedius	10.95	71.54	89.64

[1] Determined on fat- and moisture-free basis.

In studies in which the entire ham provides the source of material for investigation, a proper sampling technique is essential to the solution of the problem. The ideal sample is one from which a knowledge of the quantity and distribution of any constituent in the ham can be obtained. Consequently, a study was made with the object of developing a method of sampling cured ham that would give detailed information on salt distribution and on water distribution as well, since the latter may be valuable in explaining certain salt movements throughout the meat, particularly those accompanying the process of aging. The technique of such a method was suggested in 1936, as reported by Mohler (*19*), and has recently been applied successfully to cured lamb legs by Besley and Hiner (*3*, *4*). The present paper gives some of the detailed analytical data that prompted the use of this method.

PREVIOUS METHODS FOR DETERMINING THE DISTRIBUTION OF SALT

As early as 1911 McBryde (*16*), in a study involving sour and sound hams, attempted to determine the relative distribution of salt and potassium nitrate in hams. His sampling technique provides for a cross section about 2½ inches wide cut from the center of the ham at right angles to the femur. The ham is trimmed along the lines *lm* and *no*, as shown in figure 1, and four slices (*A*, *B*, *C*, and *D*) of approximately equal thickness and parallel to the face of the ham are removed. Slice *A* includes the face of the ham, slice *B* the bone (femur), slice *C* the meat between the bone and fat of the skin side, and slice *D* practically all fat.

Another method for determining salt distribution in hams was proposed by Lewis and outlined by Moulton (*21*, *pp. 408–409*). According to this method, a 1-inch cross-section slice is taken from the ham directly behind the aitchbone, and from the center of this slice a strip extending from the face to the skin and adjacent to the femur is removed and divided into five horizontal sections, as shown in figure 2. Using this method, Moulton presents data (*20*) indicating the salt distribution in partly cured and fully cured hams, before and after smoking. Barnicoat (*2*) and, more recently, Miller and Ziegler (*17*, *18*) and Ziegler and Miller (*24*) used a similar sampling technique. Grimes, Sewell, and Cottier (*11*) based their work on three divisions

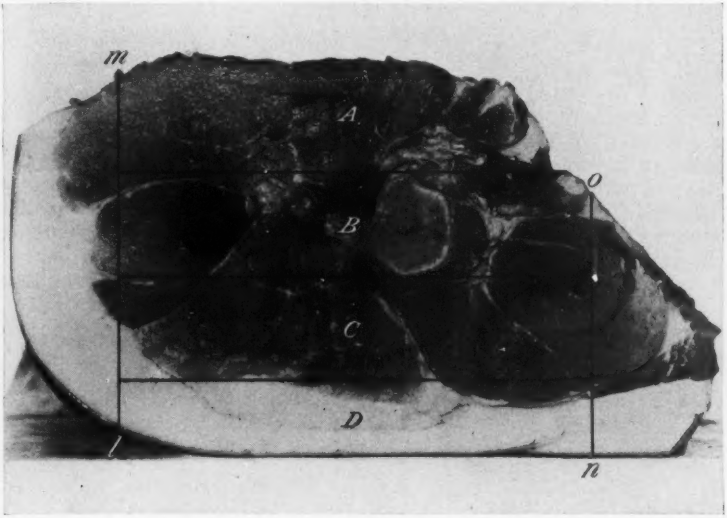

FIGURE 1.—Location of samples in cross-section slice of ham according to the sampling technique of McBryde (*16*).

of a cored sample taken crosswise through the center of the ham from the flesh side to the skin side. Apparently all these investigators used the sampling method originally suggested by Lewis or some modification of it.

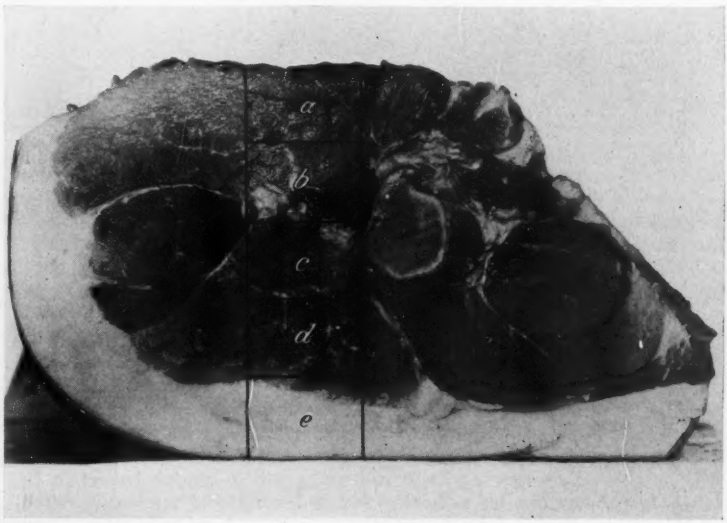

FIGURE 2.—Location of samples *a* to *e* in cross-section slice of ham cut according to the sampling technique of Moulton (*21*) and others.

PRELIMINARY STUDIES

To determine the value of these sampling techniques the following study was made. Six representative hams were removed from a batch of dry-cured hams after 46 days of curing. These hams had been cured with a mixture consisting of 8 pounds of salt, 2 pounds of sugar, and 2 ounces of saltpeter for every 100 pounds of meat. This mixture had been divided into three applications during the first 15 days of curing. Two of these hams were analyzed immediately after removal from cure, two were smoked, and two were smoked and then stored for a period of 3 months. The smoke treatment consisted of 20 hours of hardwood smoking at 110° F. Storage took place in a ham room in which the temperature fluctuated with surrounding

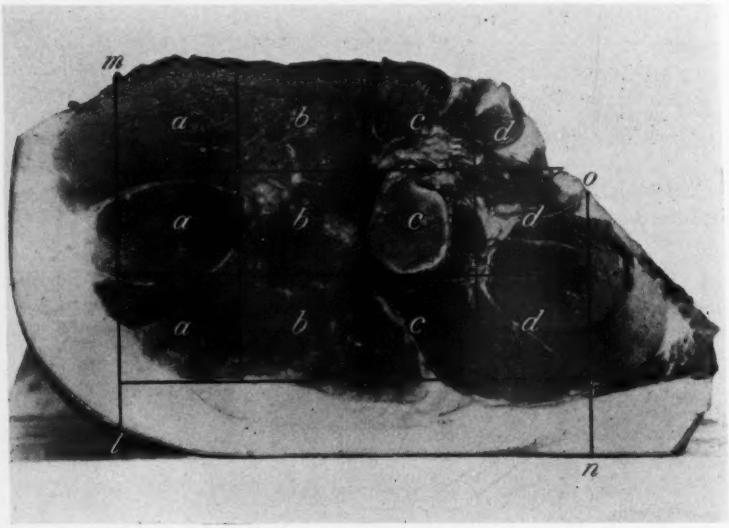

FIGURE 3.—Location of samples in cross-section slice of ham according to the sampling technique used in preliminary work by the authors.

conditions and averaged about 55° for the 3-month period. Each ham was sampled according to the technique used by McBryde (*16*), slice *D* (fig. 1) being omitted, however, since it contained little lean meat. Each of the three slices was then divided into four parts, which resulted in a grid arrangement of samples as shown in figure 3. By such an arrangement the features of the technique proposed by McBryde were combined with those proposed by Moulton (*21*), the latter method being represented by the *b* segments in figure 3, but consisting of only three instead of the four segments shown in figure 2. Barnicoat (*2*) and Miller and Ziegler (*18*) likewise omitted the fat sample from their data for the same reason that slice *D* was omitted in the present study.

After the removal of adhering fat and bone, the segments shown in figure 3 were finely ground and aliquot portions examined for their salt content by the second method suggested by Kerr (*15*) for the deter-

mination of salt in meat. In the unsmoked cured hams and those out of smoke, segments *a* and *b* were combined, as were segments *c* and *d*, in each of the three slices of the cross-section slice. In those stored for 3 months each sample was analyzed separately. The results obtained in this study are shown in table 2.

TABLE 2.—*Average salt distribution in hams after the curing, smoking, and aging processes, as determined by the McBryde (16) method of sampling, with a further division of each slice into four parts*

[All hams cured for 46 days]

Number of hams	Average weight of hams— Before any processing	Average weight of hams— After indicated processing	Stage of processing	Segments of slice [1]	Salt [2] in indicated segments of slice— A	B	C	Total
	Pounds	*Pounds*			*Percent*	*Percent*	*Percent*	*Percent*
2	14.7	14.3	46 days in cure	*a* and *b*	7.99	3.01	1.50	4.48
				c and *d*	8.81	4.62	2.85	5.59
				Total	8.40	3.80	2.25	5.04
2	16.1	14.6	Out of smoke	*a* and *b*	7.31	3.59	2.21	3.89
				c and *d*	7.43	6.45	5.48	6.45
				Total	7.37	5.09	3.41	5.06
2	15.3	12.6	3 months of storage	*a*	4.99	4.34	3.23	4.51
				b	5.96	4.23	4.73	4.87
				c	6.47	5.04	6.30	6.12
				d	3.90	8.24	7.40	6.80
				Total	5.35	5.34	5.36	5.35

[1] See figure 1.
[2] Wet basis.

The data obtained by the McBryde sampling technique (fig. 1) indicate that as a result of the curing process slice *A* contained considerably more salt than slice *B* and still more than slice *C*. A better distribution followed smoking, and at the end of 3 months of storage apparently an even distribution had been acquired. On the other hand, when each of the three slices was divided into four parts (fig. 3), the combined segments *a* and *b* consistently had less salt than the combined segments *c* and *d* at all stages of processing. Thus, the entire cross section of the hams, when sampled longitudinally by one method, had a different salt distribution than when sampled perpendicularly by another method. Therefore, the division, into 12 samples, of the material used in the McBryde technique merely emphasized to a still greater degree the lack of uniformity in salt distribution. Furthermore, neither the limits of this salt distribution nor the total salt content of the entire ham cross-section slice appeared to be adequately indicated by the *b* segments, the sampling material suggested by Moulton.

Miller and Ziegler (*17*, *18*) pointed out that equalization of salt distribution in cured hams does not become apparent until a storage period of at least 30 days has elapsed. The results in table 2 indicate that a storage period of 3 months failed to produce an equal distribution in all portions of cured hams. In further tests, several entire muscles were removed from a cured ham that had been kept at 107° F. for 20 weeks and stored for a period of 3½ years, and their salt and

moisture content determined. Results of these analyses are shown in table 3. Here, again, appears evidence of the uneven distribution of salt in hams aged for a long period. A study of tables 1 and 3 shows that the vastus intermedius muscle, which contains the most fat, contains the least salt and that the vastus lateralis muscle, which contains the least fat, is among the higher salt-containing muscles as a result of the long aging period. Although this analogy does not hold for all the muscles included in the table, it does raise again the question of the relationship between salt absorption and the chemical composition of the muscle tissue.

TABLE 3.—*Average salt and water content of nine muscles from a ham stored in ham room for 3½ years*

Muscle	Salt [1]	Water	Muscle	Salt [1]	Water
	Percent	*Percent*		*Percent*	*Percent*
Vastus intermedius	7.58	24.15	Semitendinosus	12.33	28.23
Vastus medialis	10.34	22.83	Semimembranosus	12.71	19.58
Adductor	10.35	24.00	Vastus lateralis	13.58	25.36
Rectus femoris	11.39	29.66	Gracilis	13.95	19.86
Biceps femoris	11.85	26.10			

[1] Wet basis.

STUDIES INVOLVING AN IMPROVED SAMPLING TECHNIQUE

The results of the preliminary studies indicated that neither the McBryde (*16*) technique nor that suggested by Moulton (*21*) provided sample material from which a comprehensive knowledge of the salt distribution in cured hams could be obtained. For such a study the modification of these sampling methods, as shown in figure 3, appeared to be an improvement. However, the fact that all hams are not shaped or trimmed alike makes it difficult to obtain exactly comparable samples, particularly when the method of obtaining them is based on measurements related to such variables as the size and shape of the ham. Furthermore, since variations in the composition of individual muscles may influence their ability to absorb salt as well as to lose moisture, it is desirable to distinguish between muscles in sampling. Therefore, muscle segments in the cross-section slice of the ham, as shown in figure 4, were used for sample material. The fact that in such a method the location of samples is related directly to the anatomy of the ham makes it easier to specify the location and permits a comparison of hams with assurance that the samples are from the same regions.

EXPERIMENTAL PROCEDURE

The specific technique of the proposed method of sampling is as follows. A cross-section slice is cut from the ham not more than 2 inches below the aitchbone and at right angles to the femur. The thickness of this slice is determined by the size of the ham and should be approximately one-fifth of the distance between the aitchbone and the stifle joint along the femur. The muscle segments in this slice, when carefully dissected from adhering fat and connective tissue, constitute the material for analysis. Each of these segments is cut into fine pieces with a pair of scissors, placed on tared watch glasses, and the moisture determined after heating in a drying oven

for 16 hours at 214° F. The dried material is then finely ground in a mortar and a representative sample removed for salt determination, the second method suggested by Kerr (*15*) being used. Since dried material is used for salt analyses, greater concentrations of silver nitrate and thiocyanate solutions than are recommended in the Kerr wet procedure may be necessary. The strength of these solutions depends on the quantity of the dried sample used, as well as on its relative salt content.

As a result of fat differences and various processing treatments, the moisture content of ham muscles varies slightly, thus making it necessary to express salt results on a dry basis for accurate interpretations.

Cutting up the muscle segments with scissors prior to desiccation

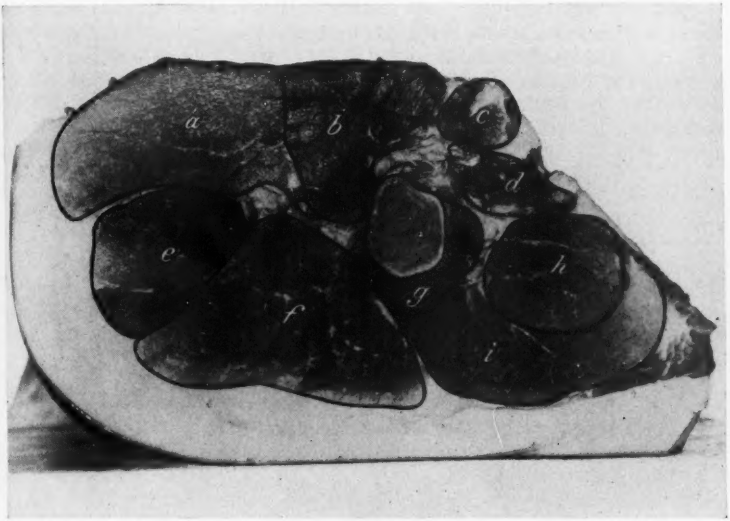

FIGURE 4.—Location of muscle segments in cross-section slice of ham according to proposed sampling technique: *a*, Semimembranosus; *b*, adductor; *c*, sartorius; *d*, vastus medialis; *e*, semitendinosus; *f*, biceps femoris; *g*, vastus intermedius; *h*, rectus femoris; *i*, vastus lateralis.

is a substitute for the usual procedure of passing the material through a meat grinder. Not only does this eliminate a somewhat laborious operation, considering the number of muscle segments used, but it results in a better mixing of the fat and lean for uniform aliquot sampling. Grinding the dried muscle segments in a mortar and pestle usually produces a well comminuted material, as the fat mixes with the lean in much the same manner as shortening is kneaded into flour.

APPLICATION OF PROPOSED METHOD TO HAMS OF VARIOUS CURES

Hams that had been cured 5, 15, and 30 days in a dry- and a brine-curing process were sampled in accordance with the proposed method.

For the dry-curing process, a mixture of 8 pounds of salt, 2 pounds of brown sugar, and 2 ounces of saltpeter per 100 pounds of meat

was used. One-half of this mixture was applied at the beginning, one-half of the remainder at the first repacking 5 days later, and the rest at the end of 15 days' curing, when the hams were repacked again. For the brine cure the same proportions of curing ingredients dissolved in 4½ gallons of water were used. The hams to be cured were covered with the brine and repacked after 5 and 15 days in the curing solution. All hams were cured at 38° F.

Several cross-section slices from each ham were obtained, as shown in figure 5, for the purpose of determining the most representative slice to be found in that portion of the ham located between the aitch-bone and the stifle joint. This is the area most commonly selected for sampling material and is considered to be fairly representative of the entire ham.

Salt and water determinations were made on those muscle segments in each slice from which sufficient material was available for analysis. Five slices were obtained from the hams cured 5 days, but the lack of sufficient muscle segments in the fifth slice resulted in the use of only four slices for sampling material in the remaining hams. The results are shown in table 4.

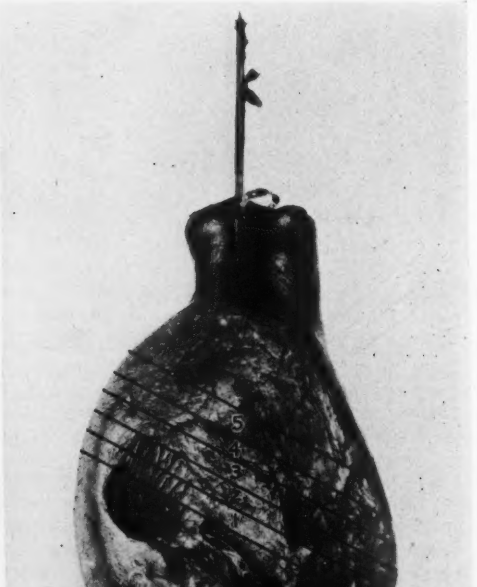

FIGURE 5.—Location of cross-section slices 1 to 5 in hams according to proposed sampling technique.

TABLE 4.—*Salt and water content of muscles in cross-section slices of hams dry- or brine-cured for various periods*

TABLE 4.—*Salt*[1] *and water content of muscles in cross-section slices of hams dry- or brine-cured for various periods*

[1 ham for each method and period of cure]

Weight of ham in pounds—		Method of cure	Period of cure	Cross-section slice No.[2]	Adductor		Biceps femoris		Gracilis		Rectus femoris		Sartorius		Semimembranosus		Semitendinosus		Vastus intermedius		Vastus lateralis		Vastus medialis		Average total slice	
Uncured	Cured				Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water	Salt	Water
					Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
				1	3.5	70.1	0.0	73.6	.....	.....	0.0	75.5	13.1	62.0	2.0	71.3	0.6	73.6	0.9	72.1	1.2	75.1	0.3	73.8	1.4	72.9
				2	.....	.....	.0	72.9	11.1	63.0	.3	75.0	11.2	63.0	2.6	70.6	.6	71.7	.3	73.3	.9	75.2	1.2	74.3	2.0	72.0
			Days	3	1.2	74.5	.6	73.5	11.7	66.1	.6	74.9	9.1	67.6	.9	73.6	.3	72.9	.0	74.6	1.2	75.5	.6	72.7	2.0	73.8
15.0	14.7	Dry	5	4	.....	.....	.9	70.6	6.1	66.1	1.5	75.1	8.2	69.5	.0	73.6	1.2	72.4	1.5	70.7	1.7	73.5	2.3	70.9	2.2	71.6
				5	.....	.....	.9	72.3	5.6	66.8	.....	.....	.....	.....	.3	74.3	1.2	74.4	.....	.....	.....	.....	.....	.....	2.4	71.5
				Average	2.5	72.1	.4	72.7	8.1	65.8	.5	75.1	10.2	65.8	1.6	72.3	.8	73.8	.7	72.7	1.2	75.1	1.5	72.3	1.9	72.5
				1	5.5	68.4	.0	74.8	.....	.....	.3	76.2	13.5	58.2	4.7	70.6	.0	74.2	.6	73.4	.3	75.7	2.0	75.5	2.0	73.0
				2	2.6	73.1	.0	73.8	10.8	65.2	.3	75.9	12.3	65.0	3.5	71.5	.6	72.7	.0	71.3	.6	76.4	.6	73.4	2.1	73.5
				3	.6	74.8	.3	72.5	11.7	64.3	.0	76.2	11.1	67.1	1.5	71.8	.9	72.0	.9	73.6	.9	76.4	1.7	74.4	1.7	73.1
15.1	15.1	Brine	5	4	.....	.....	.6	73.1	5.8	64.6	.6	76.5	7.6	68.8	.9	73.6	.3	73.6	1.2	73.1	.6	73.8	.6	73.9	1.6	72.7
				5	.....	.....	.9	71.6	5.4	62.6	.....	.....	3.2	72.5	.9	74.0	.3	73.7	.....	.....	.....	.....	.....	.....	1.6	71.7
				Average	3.8	71.2	.3	73.1	7.4	64.2	.3	76.2	10.8	65.8	2.6	72.1	.4	73.4	.6	72.9	.6	75.7	1.1	74.1	1.9	72.9
				1	10.8	68.3	1.8	66.7	.....	.....	5.6	71.4	20.7	69.7	9.4	67.2	2.3	58.4	1.5	70.9	3.5	71.9	7.0	68.6	5.2	68.3
				2	8.8	69.4	.3	64.6	15.8	68.8	6.7	76.2	17.3	71.4	8.5	66.9	2.1	58.1	1.4	71.3	1.5	72.6	4.7	63.9	6.1	68.0
				3	1.7	69.1	1.2	63.7	10.8	65.8	7.3	70.2	20.3	71.7	8.2	67.1	1.8	57.7	1.5	68.4	1.5	72.2	11.6	66.1	5.1	66.6
14.8	14.5	Dry	15	4	.....	.....	1.7	62.7	11.1	70.3	9.2	69.7	24.9	71.4	7.9	67.8	1.8	58.5	.0	72.0	.0	70.5	10.2	61.9	5.2	64.9
				Average	8.8	69.1	1.3	64.2	12.3	69.1	6.9	72.2	19.8	71.0	8.5	67.2	1.9	58.2	1.3	70.5	1.9	72.1	8.1	64.9	5.4	67.1
				1	11.1	65.4	.9	69.4	.....	.....	7.0	71.4	16.2	61.1	10.8	63.1	3.8	64.3	.9	70.8	3.2	72.0	6.7	68.8	7.0	66.7
				2	8.2	71.0	.9	69.5	16.5	62.7	8.8	70.3	20.1	64.2	7.9	67.7	1.5	61.3	.6	66.6	7.6	71.0	7.3	62.3	6.1	64.4
				3	.....	.....	1.5	63.4	14.9	62.4	11.1	67.2	21.5	64.7	4.4	70.3	1.4	61.2	1.2	67.5	2.4	70.0	7.3	61.3	4.9	66.4
14.9	15.4	Brine	15	4	.....	.....	2.0	59.6	13.2	63.7	.....	.....	.....	.....	3.8	70.3	1.2	67.1	.....	.....	.....	.....	.....	.....	4.9	66.3
				Average	9.6	68.2	1.3	65.9	14.3	63.1	8.4	70.3	19.0	63.3	7.8	66.9	2.2	63.6	.9	68.4	4.0	71.1	7.1	63.7	6.0	66.7
				1	10.6	66.8	3.4	69.2	.....	.....	7.8	68.9	27.0	70.6	13.9	67.5	3.4	67.0	2.5	72.6	9.4	71.1	8.9	66.5	8.9	68.7
				2	10.5	69.1	3.8	68.6	21.6	68.0	13.1	68.6	20.7	70.6	11.7	65.8	2.6	70.2	2.3	72.5	8.9	70.5	8.3	66.9	8.6	68.7
				3	5.4	69.4	4.1	68.2	17.4	67.9	13.2	70.3	13.7	74.0	9.0	67.9	3.2	65.1	2.9	72.0	4.4	69.9	7.7	71.2	7.9	68.7
14.7	15.2	do	30	4	.....	.....	5.3	66.2	13.7	68.3	17.1	73.6	8.2	73.4	4.9	70.2	4.2	65.5	.0	66.3	2.9	69.3	6.6	70.5	6.5	68.3
				Average	10.1	67.9	4.3	67.9	16.6	68.1	11.8	69.7	20.2	71.8	10.1	67.8	3.4	66.9	2.4	72.1	8.1	70.6	7.9	68.9	8.0	68.6

[1] Dry basis.

[2] See fig. 5.

A considerable amount of variation in salt content was found between muscle segments in the same slice, differences in water content being less marked. However, the data were obtained during the periods of maximum salt absorption in the hams, when differences in the composition and location of muscles would have a pronounced effect on salt distribution. Between cross-section slices 1 and 5 in the same ham, some muscle segments, such as the biceps femoris and rectus femoris, increased in salt content and decreased in water content rather consistently, whereas others, including the semimembranosus, gracilis, and adductor, usually lost salt and gained water. The relation of the quantity of each muscle to the total quantity of muscle examined in each cross-section slice from the hams included in table 4 is shown in table 5. Since in any one slice the biceps femoris and rectus femoris together contribute less to this total than the semimembranosus, gracilis, and adductor segments, this larger proportion of the group of muscles having a consistent loss of salt may explain the apparent decrease in salt between cross-section slices 1 and 4. The gracilis muscle appears to take up salt rapidly as the curing period lengthens, and the absence of this muscle segment from cross-section slice 1 lessens the quantity of salt in this slice. Although this muscle represents only 6.4 percent of the total muscle examined (table 5), in the earlier stages of curing it contains 30 to 40 percent of the total quantity of salt in the slices.

TABLE 5.—*Relation of each muscle segment to the average total quantity of muscle in each cross-section slice from hams included in table 4*

Muscle segment	Proportion of indicated muscle segment in cross-section slice No. —					
	1	2	3	4	5	Average total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Semimembranosus	25.0	27.2	23.4	21.8	33.2	25.0
Biceps femoris	18.2	17.1	17.0	21.8	28.6	18.8
Semitendinosus	11.7	9.9	8.9	12.4	17.4	11.0
Rectus femoris	10.7	11.6	12.6	7.8	.....	10.3
Vastus lateralis	10.9	8.6	10.1	5.0	.....	8.3
Vastus intermedius	9.3	8.4	7.6	5.2	.....	7.3
Vastus medialis	4.4	5.9	8.7	11.6	.....	7.1
Gracilis	.....	4.2	6.5	12.9	19.6	6.4
Adductor	7.8	5.0	3.2	.....	.....	3.9
Sartorius	2.0	2.1	2.0	1.5	1.2	1.9

The relation between the quantity of muscle in each cross-section slice and that in the entire portion analyzed between the aitchbone and stifle joint was determined. That a greater proportion was found in the first three slices may be seen from table 6. When five slices composed the sampling material, practically 70 percent was represented by slices 1, 2, and 3. When four slices were used the quantity of muscle in the first three slices was slightly more than 80 percent of the total sample. Furthermore the adductor muscle, which is attached to the aitchbone, does not extend sufficiently along the femur to be included in either the fourth or fifth slice. Since Hiner and Hankins (*12*) have shown this muscle to represent approximately 5 percent of all the separable lean in hams, it would appear that it should be included in any representative sampling method. The same state-

ment applies to the gracilis muscle, whose segment does not appear in cross-section slice 1. This is a thin surface muscle that covers a large portion of the face of the ham and was trimmed away close to the aitchbone in these hams.

From the evidence presented it appears that the choice of cross-section slices most representative of the ham and suitable for use in the proposed sampling technique would be one of the first three, provided all slices have the gracilis muscle. The authors selected slice 2 (fig. 5) for the reason that it usually represents the thickest and widest part of the ham and therefore surpasses other slices in total weight of lean meat. Furthermore, it contains representative segments of all the muscles between the aitchbone and stifle joint and has a salt and water content similar to that of the average slice.

TABLE 6.—*Relation of the quantity of meat in each cross-section slice to the total quantity sampled from each ham*

[1 ham for each method and period of cure]

Method of cure	Period of cure	Proportion of meat in cross-section slice [1] No.—				
		1	2	3	4	5
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry	5	21.1	26.5	23.6	21.7	7.1
Brine	5	21.8	23.8	22.8	21.4	10.2
Dry	15	22.4	28.8	27.7	21.1	.....
Brine	15	34.7	28.1	24.2	13.0	.....
Do	30	24.1	29.8	25.4	20.7	.....
Average	.....	23.5	26.7	24.3	20.4	5.1

[1] See fig. 5.

DISCUSSION

The wide differences in salt content between muscle segments in the same cross-section slice indicate the necessity of including as many of these muscles as possible in the sample to be used in determining the salt distribution in cured hams. A study of figures 2 and 4 shows that only portions of a few of these muscles are included in the sample material proposed by Moulton (*21*). These muscles are the adductor, semimembranosus, and biceps femoris, with the gracilis muscle making a fourth one if it is not trimmed away as was the case in slice 1. Although table 5 shows that these muscle segments represent more than 50 percent of the meat analyzed in any cross-section slice, only portions of these segments are included in the sample used in Moulton's method. Actually, in the hams analyzed after 3 months of storage, more than two-thirds of the total meat, which contained 70 percent of the salt, was found to be outside of the sample material used in Moulton's technique (*21*).

Although the ham-sampling method for salt determination used by McBryde (*16*) provides more of the cross-section slice for analysis, it does not take into account the fact that individual muscles located equally distant from the face of the ham may vary widely in salt content. Such differences are not distinguishable when the whole segment is analyzed as one sample. It may be argued, however, that from the standpoint of sampling, McBryde's main interest was to show that sour and sound hams did not differ in chemical analysis, and salt distribution, therefore, was not the primary part of his studies. In

this connection, the present authors applied their proposed sampling technique to several sour and sound hams that had received identical curing and smoking treatments. Their findings agreed with those of McBryde in that there was a similarity in the salt content of sour and sound hams. In addition, however, the authors' data showed that the biceps femoris and the vastus intermedius muscles contained such small quantities of salt as to exert little, if any, bactericidal action in these regions where spoilage seemed to be concentrated, despite the fact that adjoining muscle segments of equal or further distance from the face of the ham appeared to have two to three times as much salt. These results suggest that the failure of salt to penetrate in sufficient quantity into certain muscles in hams may be largely responsible for the development of spoilage in these areas, provided the spoilage organisms are present. It is also felt that McBryde would have attached more significance to ham spoilage resulting from a lack of penetration of the preserving fluids had he used a sampling method that showed more details of the salt distribution.

SUMMARY

This paper describes a method of sampling cured ham by which it is possible to obtain detailed information on salt (sodium chloride) distribution and also on water distribution. A knowledge of the latter may be valuable in explaining certain salt movements throughout the meat, particularly those accompanying the process of aging.

The results of a preliminary investigation revealed that the sampling techniques of several other investigators did not provide material from which a comprehensive knowledge of the salt distribution throughout a cured ham could be obtained.

The technique of the method proposed by the authors differs from that of the other sampling methods discussed in that the muscle segments that appear in a cross-section slice of the ham are used as the source of sample material. This material, since it is related directly to the anatomy of the ham, eliminates the use of arbitrary measurements for sampling that are based on such varying characteristics as the size and shape of the ham.

Evidence of a difference in the chemical composition of muscles of the same ham is presented, and the possible significance of this in relation to salt absorption is pointed out.

The improved sampling method was applied to several hams during the curing process. Cross-section slices taken between the aitchbone and stifle joint were obtained from each ham. Within this area five uniform slices were obtained, and, beginning at the aitchbone, the first three were found to contain about 70 percent of the total meat examined. Of the 10 muscles sampled, 4—the semimembranosus, biceps femoris, semitendinosus, and rectus femoris—contributed 65 percent of the total meat. Since slice 2 represented the thickest and widest part of the ham, contained segments of all the muscles between the aitchbone and stifle joint, and possessed an average salt and water content, this slice was selected as the most suitable for sampling purposes.

Certain practical features of the analytical procedure are discussed. Advantages pointed out consist in the cutting up of the muscle segments with scissors prior to desiccation and in comminuting segments

from muscles after the removal of water, particularly in the case of muscles containing large quantities of fat, wherein difficulty in properly mixing lean and fat in the ordinary meat grinder is often experienced.

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